

Development of freezing tolerance and vernalisation in forage grasses in field experiments (2009–2013)

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<p>Tiivistelmä — Referat — Abstract</p> <p>Finland is the northernmost cultivation area in the world and the selection of forage grass species is mostly limited by long winters and short growing seasons. Forage grasses are usually grown as mixtures of species and produced intensively for silage. The grass species most commonly used in mixtures are timothy (<i>Phleum pratense</i> L.) and meadow fescue (<i>Festuca pratensis</i> L.) which are both winter hardy species. As the climate changes in the future, more southern and more productive species like perennial ryegrass (<i>Lolium perenne</i> L.) and festulolium (<i>Festuca</i> sp. x <i>Lolium</i> sp.) may become more usable.</p> <p>The duration of snow cover has been predicted to shorten to 46 days in southern Finland by year 2050 (compared with 98 days at present). The autumns, when plants develop their tolerance against winter stresses, are also predicted to become warmer in the future. Changes in winter weather may also increase the frequency of problems such as plant exposure to freezing temperatures, associated with decreased snow cover and ice encasement due to fluctuating winter temperatures. This study presents the results of experiments carried out in Helsinki (Finland) between years 2009–2013. The experiments were done to assess the freezing tolerances and vernalisation of forage grasses and cereals hardened under field conditions.</p> <p>The vernalisation of plants was detected in all species as a decrease in days to heading during the vernalisation period. Perennial ryegrass and meadow fescue started flowering after the vernalisation was fulfilled during December-January. Winter cereals had already vernalised already in November.</p> <p>Hardening periods started at their earliest in the beginning of October. However, a deeper freezing tolerance developed during December in 2009–2010 and 2011–2012. During the winters of 2009–2010 and 2011–2012 hardening periods were long and hardening-induced temperature sums were the highest. During these winters the freezing tolerances were better in all species than during the other two winters.</p>			
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<p>iivistelmä — Referat — Abstract</p> <p>Maailman pohjoisimmalla viljelyalueella, Suomessa, merkittävin nurmien lajivalikoimaa rajoittava tekijä on talvi. Nurmia viljellään eniten lajiseoksina säilörehutuotantoon. Yleisimmät heinälajit seoksissa ovat timotei (<i>Phleum pratense</i> L.) ja nurminata (<i>Festuca pratensis</i> L.), jotka ovat molemmat hyvin talvenkestäviä. Ilmaston muuttuessa ja talvien leudontuessa potentiaalisia satoisampia lajeja voivat tulevaisuudessa olla myös englanninraiheinä (<i>Lolium perenne</i> L.) ja rainata (<i>Festuca</i> sp. x <i>Lolium</i> sp.).</p> <p>Etelä-Suomessa pakkasilta eristävän lumipeitteen keston on ennustettu lyhenevän 46 päivään vuoteen 2050 mennessä. Ennusteiden mukaan nurmien on tulevaisuudessa myös karaistuttava lämpimämmissä syysolosuhteissa kestämaan vähälumisten lauhjojen talvien tuomat haasteet, kuten kasvien altistuminen pakkasille ja maan pintaan muodostuvan jääkerroksen aiheuttamat ongelmat. Tässä tutkimuksessa esitellään Viikissä vuosina 2009–2013 tehtyjen nurmien talvehtimiskokeiden tuloksia ja tarkastellaan nurmien kylmäkaraistumista ja kukintaan virittymistä, eli vernalisoitumista vaihtelevissa syys- ja talviolosuhteissa.</p> <p>Kasvien vernalisoituminen oli kaikilla lajeilla havaittavissa kukintaan tarvittavan ajan lyhenemisenä. Englanninraiheinä sekä nurminata aloittivat kukinnan vasta vernalisaatiovaatimuksen täytyttyä joului-tammikuussa. Syysviljoilla kukintavalmius oli kehittynyt jo marraskuun aikana.</p> <p>Karaistumisjaksot alkoivat aikaisimmillaan lokakuun alussa. Syvempi kylmänkestävyys kehittyi kuitenkin vasta Joulukuun aikana. Talvina 2009–2010 sekä 2011–2012 karaistumisjaksot olivat pitkät ja karaistumista edistävää lämpösummaa kertyi enemmän kuin kahtena muuna talvena. Näinä talvina kylmänkestävyys oli kaikilla lajeilla parempi kuin talvina, jolloin karaistumisjaksot olivat lyhemmät.</p>		
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Contents

1 INTRODUCTION	5
2 FORAGE GRASSES AND CLIMATE CHANGE	7
2.1 Forage grasses in Finland	7
2.2 Overwintering	9
2.2.1 The freezing tolerance of forage grasses.....	9
2.2.2 Cold acclimation on a cellular level	10
2.2.3 The dynamics of hardening and dehardening.....	11
2.2.4 Winter damage	14
2.2.5 Vernalisation	15
2.3 Climate change.....	16
2.3.1 Climate change and changes in growing conditions in the future	16
2.3.2 The effect of climate change on agriculture	17
2.4 Overwintering models for forage grasses.....	19
3 AIMS	21
4 MATERIALS AND METHODS	22
4.1 Weather data.....	22
4.2 Experiments.....	23
4.3 Sampling, sample preparations and observations.....	26
4.4 Freezing tolerance.....	28
5 RESULTS	28
5.1 Weather and cold acclimation conditions during winters	28
5.1.1 Temperature, snow cover and precipitation	28
5.1.2 Hardening, winter and growth periods	32
5.2 Overwintering	34
5.2.1 Freezing tolerance	34
5.2.2 Heading date and final leaf number	39
6 DISCUSSION.....	46
6.1 Freezing tolerance.....	46
6.2 Vernalisation.....	47
6.3 Comparison of freezing tolerance to conditions during hardening and overwintering	48
7 CONCLUSIONS	49
REFERENCES	52

1 Introduction

Perennial forage grasses currently used in Finland have not yet been thoroughly tested for their capability to adapt to the warmer climate conditions in the future that are predicted by climate change models. It is also not known which traits are the most important for the adaptation.

Crop production occurs at higher latitudes in Finland than anywhere else in the world. In Sweden, for example, 90% of cultivation takes place at more southern latitudes than in Finland (Peltonen-Sainio et al. 2009). Nevertheless, northern regions have been considered as having potential to play an increasingly important role in European agricultural production in the future because the effects of climate change are predicted to be more positive in northern than in southern Europe (Olesen and Bindi 2002).

Finland's mild and moderately rainy climate is well suited for forage production. Forage grasses covered 28.9% of the cultivated land area (2 285 000 ha) in 2012, the majority of which (20.6%) is used for intensive grass silage production (Tike 2013). Areas of hay production, pastures, seed production and green fodder were 4.2%, 3.2%, 0.4% and 0.4%, respectively. The proportion of pasture land is low compared to southern areas of Europe. In addition, in many more southerly areas of Europe silage is often produced from maize (*Zea mays* subsp. *mays* L), which is not grown in Finland. The total fallow area, including fallows, nature management fields and grasses used for green manure, was 11.7%. The total area of grassland crops has remained relatively stable over the last two decades, although the proportion of the area used to produce silage has increased and the proportions of hay and pasture areas have decreased. The Finnish dairy production relies on local production of forage grass, and markets for silage do not exist.

Regionally tailored adaptation strategies for climate change may be necessary in the future because spontaneous adaptation, for example adoption of new cultivars and species through farmers' own decisions, has its limitations (Olesen

et al. 2011). As a solution for the adaptation of forage grass production to climate change, Rognli (2013) requested that there should be an increase in the genetic variation of locally adapted species like timothy (*Phleum pratense* L.) and breeding for improved adaptation in species like perennial ryegrass (*Lolium perenne* L.).

The length of the growing-season is predicted to increase and autumns to become warmer in the future (Ruosteenoja et al. 2010). Also, the hardening period has been predicted to take place later in the autumn (Höglind et al. 2013). The combination of good winter hardiness and effective use of an extended growing season are traits that are not present in cultivars that are currently used in northern latitude countries (Rognli 2013). The most winter-hardy species like timothy cannot use warm autumns effectively because they stop growing earlier than southern species like perennial ryegrass.

Successful cold acclimation and vernalisation are crucial for overwintering and for good regrowth in the spring. Vernalisation means the acquisition of flowering ability during the autumn in the forage grasses. The first step in the preparation of forage grasses for overwintering is the cessation of growth in the autumn. During the autumn plants also accumulate the storage carbohydrates and undergo many other physiological changes. The single most important cause of freezing damage is cell desiccation due to ice formation in the extracellular space (Bertrand & Castonguay 2003a).

Breeding for good winter tolerance has been the main goal in the breeding programmes of forage grasses for northern conditions. The goals of breeding may change in the future as winters get warmer, but the traits that affect the adaptation are still not well known. The species composition of forage grass leys in Finland may change in the future due to the predicted milder winters. The aim of this study is to inspect differences in the overwintering performance of different grass species in southern Finland based on field trials conducted in Helsinki between years 2009 and 2013. Special emphasis was on the understanding of the relationship between vernalisation and freezing tolerance in forage grasses.

2 Forage grasses and climate change

2.1 Forage grasses in Finland

Timothy is by far the most common grass species grown in Finland. It is used in forage grass mixtures together with other grasses such as meadow fescue (*Festuca pratensis* Huds.) and with forage legumes like red clover (*Trifolium pratense* L.). Timothy has good winter hardiness and it is persistent in forage grass leys even in the northernmost cultivation area in Finland. In official field trials winter damage values for timothy have been less than 10%, excluding the northernmost cultivation zone where winter damage values have been around 10–20% for most cultivars (Kangas et al. 2012b). For comparison, winter rye and winter wheat have suffered winter damage of 20–30% in southern Finland (Kangas et al. 2012b). In general, timothy cultivars can be divided into northern and southern types (Isolahti 2010). Southern types have better regrowth ability after cutting and weaker winter hardiness, whereas northern types have a poorer regrowth and a better overwintering capability (Isolahti 2010). Many cultivars have both southern and northern traits. In official field trials a southern-type cultivar Grindstad has produced the highest total dry matter yields and has still managed to overwinter successfully, even in the northernmost cultivation zone with winter damage values of 20% (Kangas et al. 2012b).

Meadow fescue and tall fescue (*Festuca arundinaceae* Shreb.) are also well adapted for cultivation in Finland. Meadow fescue is often used due to it having better regrowth ability than timothy. Cultivation of tall fescue has increased, mostly at the expense of meadow fescue, in the last few years due to its better drought tolerance and regrowth ability and its higher yield as shown in field trials (Kangas et al. 2012a). There are challenges in management practices with tall fescue, however, such as timing of the optimal harvest in mixtures.

Perennial ryegrass is an important forage grass in warm temperate zones but it suffers from substantial winter damage. Two cultivars, Riikka and Svea, have been studied in official field trials and their winter damage values have been around 10–20% in southern Finland (Kangas et al. 2012b). Perennial ryegrass is

especially susceptible to damage caused by ice encasement and moulds (Kangas et al. 2012a). Therefore, yields of perennial ryegrass often decline substantially in three-year-old leys due to extensive winter damage. Annual Italian ryegrass (*Lolium multiflorum* Lam.) is rarely used in Finland, although it can be used as an annual monoculture which gives a usable forage yield till late autumn due its annual growth habit.

Festulolium is a hybrid between either meadow or tall fescue and perennial or annual ryegrass. The two festulolium cultivars that have been tested in official field trials in Finland have suffered extensive winter damage in two northernmost cultivation zones and thus its cultivation has been recommended only for southern and mid Finland (Kangas et al. 2012b).

Timothy has traditionally been cut two times during the growth period in Finland, and it has not benefited from a three-cut strategy like meadow and tall fescue and festulolium (Kangas et al. 2012b). Differences in the yield increases can partially be explained with different growth habits because timothy's regrowth occurs through basal buds whereas the fescues and ryegrass regrow from small vegetative tillers. These different regrowth strategies, and thus different cutting strategies, need to be taken into account when mixtures are chosen. Late autumn cutting can have an effect on the success of winter hardening of forage grasses and, therefore, the latest recommended cutting time is four to five weeks before the end of the growth period or after the growth has ended. Farming practices should always be timed so that the cold-acclimation processes can proceed successfully.

2.2 Overwintering

2.2.1 The freezing tolerance of forage grasses

Successful cold acclimation which leads to improved freezing tolerance and accumulation of reserve carbohydrates is crucial for winter survival. The overwintering parts of forage grasses are protected from freezing, for example by breaking down storage carbohydrates and lowering the osmolarity of cells. In addition, the overwintering parts are near or at ground level, where even a little snow cover provides protection from freezing damage. Temperature fluctuations are much lower in the soil than in the air and therefore plants are not exposed to an equally severe temperature stress at ground level, especially when snow cover is present. For example, timothy forms overwintering bulb-like structures at the base of the tillers at ground level. Tillering in timothy occurs through the buds on these bulb-like structures.

Acclimation to cold starts in plants with growth cessation in the autumn and with reserve carbohydrate and dry matter accumulation which lead to removal of water from the intracellular space (Bertrand & Castonguay 2003a). During freezing stress, water is drawn from the intracellular space to the extracellular space where ice crystals form. However, if the freezing stress is too severe or lasts too long, excessive dehydration occurs and cell collapse occurs (Pearce et al. 2001). Partially dehydrated tissues, for example seeds, are usually the most-tolerant tissues to freezing temperatures because the amount of freezing water that causes damage is limited. Dehydration of cells can result in the concentration of solutes in the cell sap becoming too high, which results in problems in metabolism. Before this potentially fatal situation is reached, the exposure to low temperatures generally increases the cells' tolerance to the stress due to concentrated cell solutes (Andrews 1987).

The freezing tolerance of plants can be measured by exposing them artificially to a series of low temperatures, and then observing the temperature at which 50% of the plants are killed. This is done by using viability experiments where plants

are grown in a greenhouse and the viability of the tillers is observed visually (Larsen 1978). The viabilities at different temperatures can be used to calculate the LT_{50} value. Measurements are done by exposing the plants to freezing temperatures for a short time; therefore, the LT_{50} values cannot be thought of as absolute freezing tolerance temperatures in field conditions, where the exposure to freezing temperatures can occur for considerably longer periods (Gusta et al. 2003). However, they can be used to compare the genotypic differences in freezing tolerance (Fowler et al. 1981).

2.2.2 Cold acclimation on a cellular level

Cold acclimation (hardening) is a process where low but non-freezing temperatures and short day length induce plants to undergo several biological changes in order to achieve the required frost tolerance (Sakai and Larcher 1987). Temperatures inducing cold acclimation are typically between 0 and 10 °C. These biological changes include changes in non-structural sugar contents, changes in the photosynthetic machinery and accumulation and degradation of proteins (Sandve et al. 2011). Increased photoinhibition, accumulation of anti-freeze proteins and fructan synthesis are the main factors in cold acclimation processes (Krause 1988, Sandve et al. 2011). The maintenance of membrane functions at low temperatures is also crucial for overwintering plants, and therefore plants have developed mechanisms like alteration of lipid composition in the cell membranes (Bertrand & Castonguay 2003a).

Photoinhibition is a situation where the energy demand of light-independent reactions is exceeded by light energy absorbed in the photosynthetic processes, which leads to destruction of the photosynthetic apparatus or production of deleterious reactive oxygen species (Krause 1988). Plants differ in their ability to tolerate cold-induced photoinhibition. A relationship between tolerance to cold-induced photoinhibition and freezing tolerance in winter wheat and winter rye has been found (Huner et al. 1993).

The formation of anti-freeze proteins, especially ice re-crystallisation inhibition proteins (IRIPs), is thought to be an important part in avoiding the damage done by apoplastic ice crystal formation (Griffith et al. 2005). IRIP proteins induce the formation of small ice crystals instead of the normal large ice crystals which draw water from the symplast more efficiently. Meadow fescue's IRIP genes transferred to *Arabidopsis* have been shown to have an effect on freezing tolerance and cell membrane stability, which supports the evidence for the importance of anti-freeze proteins in overwintering temperate grasses (Zhang et al. 2010). Direct and detailed studies of their importance in the development of freezing tolerance in forage grasses are still missing, however.

Fructans, soluble fructosyloligosaccharides, are carbohydrate storage molecules which have a role in freezing and low temperature stress tolerance (Vijn & Smeekens 1999, Livingston et al. 2009). Breakdown of fructans decreases the osmotic potential of cells and decreases the freezing point, and thus decreases the amount of freezing water. Hisano et al. (2004) have also shown that fructans stabilize cell membranes and thus improve freezing tolerance in perennial ryegrass. Forage grasses adapted to warmer climate usually accumulate starch in chloroplasts, and those adapted to cold winter conditions accumulate large amounts of fructans in vacuoles (Pollock 1991). In timothy and perennial ryegrass non-structural carbohydrate accumulation differs depending on the plant's developmental stage and genetics (Østrem et al. 2011). Isolahti et al. 2004 did not find a correlation between fructan content and LT₅₀ values in timothy, but due to the breakdown of fructans there was a significant positive correlation between total carbohydrate content and the total non-structural carbohydrate content.

2.2.3 The dynamics of hardening and dehardening

Temperate grasses and winter cereals cold acclimate during the autumn to resist the physical and physiological stresses that occur during the winter. Freezing, fluctuating temperatures, duration of snow cover, ice encasement and waterlogging are all factors that affect the winter survival of forage grasses and winter cereals (Bertrand et al. 2003b). The plant's ability to resist these stresses

is affected by the conditions and the length of the hardening period prior to winter (Hofgaard et al. 2003, Jørgensen et al. 2010, Rognli 2013).

The LT_{50} value means the temperature that is lethal to 50% of the plants in controlled freezing tests. The freezing tolerance of plants is a dynamic trait that fluctuates together with the ambient temperature, but the magnitude of the response is cultivar and species dependent (Bergjord et al. 2008, Jørgensen et al. 2010). Hulke et al. (2008) studied perennial ryegrass and found a close association between cultivars' winter survival in the field and the freezing tolerances of plants hardened in controlled conditions. Therefore, they proposed that freezing tolerance can be a useful measure for a cultivar's true winterhardiness in field conditions when there is little stress. Strong correlation between freezing tolerance and field survival has also been found for winter wheat (Fowler et al. 1981). The observed maximum freezing tolerances (lowest LT_{50} values) often vary considerably between experiments (Table 1).

Table 1. LT₅₀ values of forage grasses in different experiments.

Location	Species (cultivar), LT ₅₀	Description	Cited author
Finland	Timothy (25 genotypes) -13.8 to -21.8 °C (mean 18.1 °C)	Plants were grown and hardened in controlled conditions. Freezing tests done in cold bath.	(Isolahti et al. 2004)
Finland (Viikki)	Timothy (1993) cv. Bilbo -9.9 cv. Iki -12.2	Plants were grown and hardened in pots in field conditions. Freezing tests done in cold bath.	(Kananen 1996)
Norway (Holt)	Timothy cv. Engmo <-26.0 (2005–2006) -26.0 (2006–2007) cv. Grindstad -20.0 (2005–2006) -19.7 (2006–2007) Perennial ryegrass cv. Riikka -13.2 (2005–2006) cv. Gunne -11.2 (2005–2006)	Plants were grown and hardened in field conditions. Freezing tests done in programmable freezer and with tillers in moist sand.	(Jørgensen et al. 2010)
Norway (Særheim)	Timothy (mean of 2 cultivars) -19.9 (2005–2006) -17.6 (2006–2007) Perennial ryegrass (mean of 2 cultivars) -12.2 (2005–2006) -9.8 (2006–2007)	Plants were grown and hardened in pots in field conditions.	(Höglind et al. 2008)
US	Perennial ryegrass (21 genotypes) -10.3 to -14.0 °C	Plants grown and hardened in controlled conditions. Significant correlation was found between LT ₅₀ and tiller survival in the field. Freezing tests done in programmable freezing chamber and with tillers in growing medium.	(Hulke et al. 2008)

Dehardening (de-acclimation) is a process which initiates when plants experience a sufficiently long and warm period during or after winter and therefore restore their ability to start growing (Thorsen et al. 2010a). Overall, dehardening and rehardening are complex processes affected by factors such as carbohydrate storages and metabolism, water availability, ambient temperature, photoperiod, growth and development and the dormancy state of the plant (Kalberer et al. 2006). Dehardening can be either reversible or irreversible. The elongation of tillers due to a high temperature, day length or fulfilment of vernalisation requirement results in irreversible dehardening (Olesen et al. 2011).

The rehardening ability of plants is thought to be an alternative or at least a supplementary strategy for frost injury avoidance during the fluctuating freeze-thaw cycles (Kalberer et al. 2007). The rehardening ability of timothy declines during the winter towards the spring (Thorsen et al. 2010a). Timothy is known to be more resistant to dehardening under ice than perennial ryegrass (Höglind et al. 2010).

2.2.4 Winter damage

Biotic stress factors such as snow mould pathogens are the most important causes of winter damage for forage grasses in the inland regions of northern Europe, whereas in coastal areas winter damage is mainly caused by frost and ice cover (Höglind et al. 2013).

Ice encasement is a problem in conditions where repeated freeze-thaw cycles form an ice layer on the ground. Solid ice sheets can cause near anoxic conditions in the soil due to their almost total impermeability to gas diffusion and can thus be even more deleterious to the plants than waterlogged soils (Höglind et al. 2010). The texture of the ice may also increase survival of the plants if it has enough pores or air passages to inhibit the formation of anoxic conditions (Andrews 1987). Ice encasement tolerance correlates linearly with freezing tolerance in timothy and perennial ryegrass (Höglind et al. 2010). Plants can also suffer damage due to capillary formation of ice in the uppermost few centimetres of the soil, a process which raises plants and breaks the roots.

Plants that have evolved to overwinter under near anoxic conditions under ice sheets or in waterlogged soils have an ability to reduce or at least maintain a stable glycolytic rate during the period of oxygen shortage (Bertrand et al. 2003b). Timothy is categorized among one of the most ice-encasement tolerant species and perennial ryegrass among one of the least tolerant forage grass species in this regard (Höglind et al. 2010). Timothy maintains high amounts of carbohydrates in anoxic conditions (Bertrand et al. 2003b). Winter wheat and some temperate grass species have been shown to carry out photosynthesis in ice encasement and sub-zero temperatures, which may also affect the declining rate of frost tolerance (Andrews 1989). The minimum temperature at which photosynthesis has been shown to occur is -4 °C in perennial ryegrass and tall fescue, and many other pasture grass species, and the minimum photosynthetic CO₂ uptake temperature does not seem to differ much between the species (Skinner 2007, Höglind et al. 2011).

2.2.5 Vernalisation

Vernalisation is a process where plants obtain their flowering ability during exposure to cold but non-lethal temperatures. Overwintering temperate cereals, like winter wheat and winter rye, require vernalisation before they can initiate flowering (Chouard 1960). Some forage grass species, like meadow fescue and perennial ryegrass, also require vernalisation to initiate flowering (Heide 1994, Fowler et al. 1999). Timothy does not have an obligatory vernalisation requirement like winter cereals (Heide 1994), but the formation of generative tillers initiates earlier in vernalised than in non-vernalised plants (Seppänen et al. 2010). In winter wheat there is a link between hardening and vernalisation time; non-vernalised plants harden at a faster rate than vernalised plants (Fowler et al. 1999). In the course of vernalisation time to flowering, i.e. heading date (HD) gets shorter and final leaf number (FLN) lower as the transition from vegetative to generative stage progresses (Mahfoozi et al. 2001). When the decrease in FLN after different vernalisation time is levelled off, vernalisation requirement is thought to be fulfilled. The FLN of the main stem measures when the main tiller's

apex has changed from vegetative to generative form, based on the assumption that leaves are produced on a linear rate. The effects of day length and temperature on the final leaf number have been well studied in wheat (Baker and Gallagher 1983, Mahfoozi et al. 2001). In winter cereals the photoperiod sensitivity of plants does not affect the timing of vernalisation saturation (Mahfoozi et al. 2001). However, in short day sensitive wheat, short day length extends the acclimation period and improves the freezing tolerance (Mahfoozi et al. 2001).

Vernalisation of perennial forage grasses and winter cereals can have a great impact on the canopy structure and tiller composition in some species during the spring growth, especially timothy (Seppänen et al. 2010, Virkajärvi et al. 2012). The vernalisation time of timothy seems to have an optimum, after which the extended exposure to vernalising conditions results in a decrease in the number of generative tillers (Seppänen et al. 2010). Although vernalisation often occurs simultaneously with low temperature induced cold acclimation, it has been shown that there exist distinct pathways which control these two processes, at least in *Arabidopsis* (Bond et al. 2011).

2.3 Climate change

2.3.1 Climate change and changes in growing conditions in the future

Climate is warming globally and over the period of 1880–2012 the global combined land and ocean surface temperatures have increased by 0.85 °C (IPCC 2013) and at high latitudes the temperatures are expected to rise more than anywhere else (IPCC 2013). In the last two decades the climate has been warming at a faster pace than ever before in the period for which there is accurate measurement history (IPCC 2013). The thermal winter will likely cease to exist in the southernmost Finland by the end of this century and the temperature conditions will resemble those currently occurring in central Europe (Ruosteenoja et al. 2010). The winters in northern Finland will become similar to those currently experienced in southern Finland (Ruosteenoja et al. 2010). The thermal winter

will also shorten more rapidly than the rate at which the thermal summer will lengthen (Ruosteenoja et al. 2010).

Under high emission scenarios, a large decrease in the length of snow covered period from 1960–1990 to 2050 is predicted for southern Finland (Jokioinen): from 142 to 46 (decrease of 96 days) snow covered days (Höglind et al 2013). In northern Finland (Rovaniemi) the change is predicted to be much smaller: from 193 to 167 (decrease of 26 days) snow covered days. The number of frost days may decrease to under 100 in Finland's south-western archipelago by 2025 and elsewhere in southern Finland by 2055 (Peltonen-Sainio et al. 2009).

The amount of precipitation at high latitudes is also expected to increase in projections (IPCC 2013) and it is almost certain that precipitation will increase in winter, spring and autumn (Ruosteenoja et al. 2010). With high emission scenarios, precipitation will increase by 0–16% in northern Europe by 2080, while in southern Europe precipitation is predicted to decrease by 4 to 27%. In addition, daily temperature and precipitation extremes are expected to become more frequent in the future (Klein Tank et al. 2003). Rainless periods are also predicted to become longer in the future (Ruosteenoja et al. 2007).

2.3.2 The effect of climate change on agriculture

Crop production in Finland is mainly limited by the short growing season (Peltonen-Sainio et al. 2009), although in recent years the growing season has already become longer and warmer (Carter 1998, Klein Tank et al. 2002). The start of the thermal growth period has been predicted to occur earlier in Finland by 2050 compared to the period of 1960–1990, although the change is thought to be smaller than in other regions of northern Europe (Höglind et al. 2013). In southern Finland (Jokioinen) the predicted change in the average start date of the thermal growth period is from 8.5. to 27.4. (11 days) and in northern Finland (Rovaniemi) from 26.5. to 16.5. (10 days). The thermal growth period will likely be extended by 35 to 55 days by the end of the century depending on which emission scenario is used in projections (Ruosteenoja et al. 2010). When the high

emission scenario is used, climate-change projections for southernmost Finland, including Helsinki, are for a likely increase in the effective temperature sum (above 5 °C) from the current (1971–2000) 1200 °Cd to 1600 °Cd by 2040–2069, and to almost 2000 °Cd by the end of the century (Ruosteenoja et al. 2010).

The beginning of the hardening period is predicted to take place later in the autumn in Finland in 2050 when compared with the long-time average (1960–1990) (Höglind et al. 2013). In Jokioinen the predicted delay in the beginning of hardening period is 16 days (from 15.10. to 31.10.) and in Rovaniemi 9 days (from 29.9. to 8.10.) (Höglind et al. 2013). The lengths of hardening periods are predicted to shorten but, at least in Norway, they will still be long and cold enough to induce hardening deep enough for forage grasses to overwinter successfully (Thorsen and Höglind 2010b). For comparison, in eastern Canada hardening periods are predicted to become four days shorter by 2040–2069, and with less accumulation of hardiness-inducing temperatures (decrease in FH-COLD value) (Bélanger et al. 2002).

A temperature rise of above 2 °C from the preindustrial average will likely have a negative impact on crop production globally (IPCC 2007). In northern European conditions elevated temperatures have mostly decreased the yields of field crops (Peltonen-Sainio et al. 2011). The elevated temperatures may benefit grass crops more than other crop species (Hakala & Mela 1996).

The increased temperature in the winter period due to climate change has been thought to lead to better overwintering conditions for southern forage grass genotypes (Höglind et al. 2013). In Finland, however, that may not be the case, because here the risk of frost damage during winter period is predicted to increase for both timothy and perennial ryegrass (Höglind et al. 2013). In addition, winter damage to forage grasses due to other modelled abiotic stress factors have been predicted to rise in southern Finland, but in most areas in northern Europe the risk of winter damage is not predicted to change (Höglind et al. 2013). In most regions damage is mainly prevented because in the future temperatures will not go as low as in the past, although the minimum freezing tolerance stays higher than before (Höglind et al. 2013). In eastern Canada the decrease in snow

cover in the future has been predicted to result in increased winter damage of forage grasses due to dehardening, ice encasement and exposure to freezing temperatures (Bélanger et al. 2002).

The predicted increase in the amount of precipitation in the autumn (Jylhä et al. 2004) may prevent the benefits of the extended harvesting time due to the extended growth period (Peltonen-Sainio et al. 2009). Even so, the wider use of overwintering cereals, especially winter wheat and probably also winter barley and winter oat, may become appropriate due to their higher yield potential and their ability to avoid summer and spring droughts because of their more developed root system (Peltonen-Sainio et al. 2009). The increased production risks and uncertainty, due to weather extremes, more varying growing conditions and outbreaks of pests and diseases, may have a negative effect on the interest of farmers to grow more sensitive plants in higher latitudes (Peltonen-Sainio et al. 2009).

2.4 Overwintering models for forage grasses

Modelling the damage to forage grasses during winter can be done by using factors and agroclimatic indices that describe the dynamics of winter damage and growth during autumn, winter and spring. Overwintering models have not been used for extensive assessment of how forage grasses react to changed overwintering conditions in the future in Finland.

Recently in Norway, Höglind et al (2010) developed and validated an overwintering model of timothy for Norwegian conditions that had originally been developed for eastern Canadian conditions by Bélanger et al. (2002).

The model by Bélanger et al. (2002) included the following indices: FH-COLD, FH-RAIN, W-THAW, W-RAIN and W-COLD (table 2). FH-COLD is a net accumulation of hardening inducing cold degree-days (between 0–5 °C) during the hardening period before the occurrence of potentially damaging freezing temperatures. The selected temperature in the FH-COLD index is based on the

assumption that the transition between growth and hardening processes accelerate at 5°C in forage grasses. FH-RAIN and W-RAIN indices describe the daily amount of precipitation during the hardening period and winter period, respectively. W-THAW describes the amount of thawing conditions during the winter period by calculating the accumulation of degree days using 0°C as a base temperature. W-COLD is an index that describes a plant's exposure to freezing temperatures by calculating the difference between the number of days with snow cover (≥ 10 cm) and the length of the cold period when temperatures ≤ -15 °C.

Thorsen and Höglind (2010b) adapted the agroclimatic indices of Bélanger's model for Norwegian conditions. The FH-RAIN index was excluded from the model due to inconsistent results on the effect of rainfall on hardening in Norway. Instead, they used the following new indices: AF (autumn frost), WF (winter frost), ID (ice damage) and SF (spring frost). Frost indices (AF and WF) are used to estimate the number of days with lethal temperatures during the hardening and winter periods based on the frost-tolerance model developed by Thorsen and Höglind 2010a. The frost-tolerance model estimates the number of days when the simulated LT_{50} value exceeds the daily minimum air temperature (Thorsen et al. 2010). Ice damage index describes the formation of ice encasements on the field surface based on the SnowFrostIce model (Thorsen and Höglind 2010a). Spring frost (SF) is an index which describes the occurrences freezing temperatures after the start of the thermal growth period which can kill the dehardened plants (Gay et al. 1991).

Thorsen and Höglind (2010b) compared four different frost tolerance (LT_{50}) models for timothy by comparing the outcomes of simulations and observation data from Norway. The snow cover, soil frost and soil surface temperature were simulated in all cases by the SnowFrostIce model (Thorsen and Höglind 2010a). The four compared models were a process based model LINGRA for timothy, FROSTOL model for wheat (Bergjord et al. 2008) and two simplified versions of the FROSTOL model. They concluded that a simplified model of FROSTOL was the most suitable for modelling the hardening and dehardening processes of timothy in the future. This model may need a calibration, however, when used with different timothy cultivars due to the genotypic differences in the winter

hardiness of the cultivars. The simplified FROSTOL model has recently also been adapted and used to assess uncertainties in impact of climate change on forage grass production in northern Europe, including Finland (Höglind et al. 2013). Simulations were carried out for three locations in Finland: Rovaniemi, Kuopio and Jokioinen.

The models have not been used to assess the hardening and dehardening processes and the extent of winter damages for the coastal region of Finland, including Helsinki. In addition, no calibration data for the hardening processes of forage grasses exist for that region.

3 Aims

The aim of this study was to examine the development of overwintering processes of forage grasses in the field during the four winters between 2009 and 2013 in Viikki (Helsinki, Finland). Data on freezing tolerance and vernalisation were compared between selected species to describe their differences in response to varying hardening and overwintering conditions. In addition, hardening and vernalisation processes were compared with the describing factors of the autumn and winter conditions (Table 5). The data for forage grasses were also assessed against the data collected on winter cereals, for which there are more reports of studies in the literature than there are for forage grasses.

The following hypotheses were used to assess the differences in the performance of different forage grass species and cultivars:

1. The freezing tolerance (LT_{50} value) of plants is affected by the genetic background of the plant, i.e. the freezing tolerance differs between the species within winters.
2. The development of freezing tolerance (LT_{50} value) is affected by the temperature during the hardening period.
3. The hardening period has an effect on the timing of vernalisation processes, i.e. vernalisation occurs at different times between the years.

4. The genetic background of the plant has an effect on the timing of vernalisation fulfilment.

4 Materials and methods

4.1 Weather data

Weather data for the study period of 2009–2013 were received from The Finnish Meteorological Institute (FMI). The weather station (ID0339, WMO02998, 60,20N, 24,96E) was located in Kumpula (Helsinki, Finland) approximately 4 km from the field trials of Viikki and at an elevation of 24 m above sea level. The data included daily mean and daily lowest temperature, daily precipitation and snow depth. Each year was divided into growth, hardening and winter periods. Different describing factors of weather and growth conditions for these periods were calculated (Table 2) according to methods proposed by Bélanger et al. (2002) and Thorsen and Höglind (2010b). These factors were compared with the timing of vernalisation and hardening processes. Winter periods were calculated according to Bélanger et al. (2002) because calculations of winter period according to Thorsen and Höglind (2010b) would have required the simulation of LT_{50} value for the determination of the start of the winter period. Precipitation data for the beginning of the winter period in winter 2009–2010 were not available so the mean daily precipitation was calculated using only the available data from the end of the winter period. W-THAW index was not calculated because soil temperature data for the winters were not available. The soil temperature for winter 2011–2012 was recorded from the winter cereal field trial using a Decagon Em50 data logger with five temperature sensors at a depth of ~2 cm.

Table 2. Calculations of variables and indices for autumn, winter and spring. Abbreviations: HP (hardening period), GP (growth period), WP (winter period). Calculations were done using daily mean air temperatures. (Bélanger et al. 2002, Thorsen and Höglind 2010b)

Variables and indices	Equations	Descriptions
A (HP)		Number of days between G1 (HP start) and G2 (HP end)
B (DD5)	$\sum_{i=G1}^{G2} DD5i$, where DD5 is degree days using 5 °C as base temperature.	DD5 sums during hardening period
C (CDD5)	$\sum_{i=G1}^{G2} CDD5i$, where CDD5 is degree days of days when $0 < T < 5$ °C	CDD5 sums during hardening period
D (FH-COLD)	$\sum_{i=G1}^{G2} CDD5i - \sum_{i=G1}^{G2} DD5i$	Net accumulation of CDD5 during hardening period
D2 (W-RAIN)	$\frac{\sum_{i=F1}^{F2} \text{Daily precipitation (mm)}}{H}$	
E1 (GP start)		Last day of first five day period when daily mean air temperature ≥ 5 °C
E2 (GP end)	G1-1	Date prior to G1 (HP start)
F1 (WP start)		Date of first occurrence of minimum air temperature ≤ -15 °C
F2 (WP end)		Date of last occurrence of minimum air temperature ≤ -15 °C
G1 (HP start)		Date following last day when C (CDD5 sum) = 0
G2 (HPend)		Date of first occurrence of daily minimum air temperature < -10 °C
H		Number of days between WP start and WP end

4.2 Experiments

The data sets were collected during the four winters between years 2009 and 2013. Data were collected from two consecutive field trials conducted in Viikki (Helsinki, Finland) in fields of the University of Helsinki's research farm. All field trials were carried out as a randomized block design with 4 blocks and one plot

per cultivar. Plants for all field trials included cultivars or breeding lines representing different origins, i.e. southern and northern genotypes. The first field trial for winters 2009–2010 and 2010–2011 included 8 timothy cultivars (Table 3). The first field trial was sown in Viikki (60,13N 25,01E at an elevation of 13 m) with barley (*Hordeum vulgare* L.) as a cover crop on 22.5.2009. Details of the establishment and management of the first field trial are available in Luhtanen's Master's thesis (2011). The second field trial was established in Viikki on 30.6.2011 without the cover crop. Species included in the second field trial were timothy, meadow fescue and perennial ryegrass (Table 3). The plots were fertilized at establishment with approximately 20 kg N/ha. No additional management was carried out during the summer or autumn of 2011. In 2012 the canopy was cut on 12.6.2012 and fertilized on 20.6.2012 with 90 kg N/ha (Suomensalpietari N-P-K-S 27-0-1-4). The second cut was not done in summer 2012 due to high amount of precipitation, but in the autumn the grass was cut to a residual sward height of 10 cm on 12.10.2012.

For comparison, samples were collected from winter cereal field trials during the winters 2011–2012 and 2012–2013. These field trials were also located in Viikki (Helsinki, Finland) (60,13N 25,02E at an elevation of 12 m) and they included two winter wheat cultivars (Olivin, Magnifik) and two winter rye cultivars (Kier, Riihi). In the first year the winter rye was sown on 19.8.2011 and winter wheat on 7.9.2011 with 500 seeds/m². The field was fertilized with 20 kg N/ha (ChemAgro N-P-K 16-7-13). The field trial for the second winter was sown on 31.8.2012 with 500 seeds/m² and fertilized with 30 kg N/ha using Cemagro (N-P-K 28-3-5).

Table 3. Forage grass species and cultivars or breeding lines used in the field experiments during 2009–2013. Measurements and observations conducted during each winter period are shown.

Winter	Species and cultivars (cv.)/breeding lines (b.l.)	Measurements and observations
2009–2010	Timothy (<i>Phleum pratense</i> L.) cv. Tuure cv. Iki cv. Tammisto II b.l. Bor01025 cv. Grindstad cv. Jonatan cv. Tika cv. Tia	HD Viability/LT ₅₀
2010–2011	Timothy (<i>Phleum pratense</i> L.) cv. Tuure cv. Iki cv. Tammisto II b.l. Bor01025 cv. Grindstad cv. Jonatan cv. Tia cv. Tika	FLN (only timothy) HD Viability/LT ₅₀
2011–2012 & 2012–2013	Timothy (<i>Phleum pratense</i> L.) cv. Tuure cv. Grindstad b.l. Bor0307 b.l. Bor0113 b.l. Bor01025 b.l. Bor88060 Meadow fescue (<i>Festuca pratensis</i>) cv. Ilmari b.l. Bor20613 b.l. Bor20614 Perennial ryegrass (<i>Lolium perenne</i> L.) cv. Riikka Winter wheat (<i>Triticum aestivum</i> L.) cv. Magnifik cv. Olivin Winter rye (<i>Secale cereale</i> L.) cv. Riihi cv. Kier	FLN* HD Viability/LT ₅₀

*Final leaf number of winter cereals was observed only during the winter 2012–2013.

4.3 Sampling, sample preparations and observations

Plants were collected from the field trials once a month during the winter to test their freezing tolerance, heading dates (HD) and final leaf number (FLN) in a greenhouse (Table 4). In winter 2009–2010 samples were collected in November, December, January, April and May. Samplings in February and March were not done due to the snow cover being too deep. In winter 2010–2011 sampling was performed once a month from October to May. During the winters 2011–2012 and 2012–2013 forage grass samples were collected from October to March. Cereals were sampled during winter 2011–2012 from November to April and during winter 2012–2013 from November to March.

Table 4. Sampling dates. F = forage grasses and C = cereals.

Winter		October	November	December	January	February	March	April	May
2009	F	-	21.11.	16.12.	25.1.	-	-	26.4.	24.5.
2010	F	27.10.	23.11.	27.12.	24.1.	21.2.	21.3.	18.4.	23.5.
2011	F	24.10.	20.11.	20.12.	31.1.	28.2.	-	3.4.	-
	C	-	28.11.	-	3.1.	6.2.	5.3.	-	-
2012	F	31.10.	-	3.12., 31.12.	29.1.	25.2.	25.3.	-	-
	C	-	5.11.	10.12.	9.1.	7.2.	6.3.	-	-

Only one plot per cultivar was used throughout the winter to collect field samples. This procedure was for practical reasons. If snow was present it was removed with a shovel and after sampling the pit was covered with snow again. The sampling area was approximately 1 m² and samples were dug out either with a shovel or a pickaxe depending on how frozen the soil was at that time. Samples were collected as ~10 cm blocks from the row with surrounding soil material to prevent roots from breaking. Three to six blocks were collected depending on the row's tiller density. Samples were immediately transferred to an unlit cold room at 4 °C for thawing (one day).

After thawing, the plants were washed clean from soil with cold water and the main tillers were separated. The roots of tillers were cut to a length of ~3 cm and leaves to ~5 cm before putting them into test tubes as bundles of four tillers. In winter 2012–2013 a small piece of damp paper towel was put to the bottom of the test tubes to ensure uniform freezing. Washing and separation of the tillers was performed at room temperature and thus, the plants were exposed to room temperature for three to seven hours depending on the amount of samples. Afterwards, the test tubes were immediately transferred to a cooling bath (LAUDA Proline RP3530) (0 °C) for 14–18 hours and the control plants in similar tubes to an unlit refrigerator (4°C). The next day the cooling bath was programmed to lower the temperature by 2.5°C per hour from 0 to -17.5°C, after which the temperature was lowered by 5.0°C per hour to the lowest test temperature; this varied between months from -20.0 to -27.5°C. This was not thought to result in different LT₅₀ values than slower freezing, based on Höglind et al. 2010. The cultivar test tubes were taken out of the cooling bath at 2.5°C intervals to a refrigerator (4°C). The plants were kept in the refrigerator covered with aluminium foil until transplanting to the greenhouse on the following day.

Plants were transplanted to growth peat (Kekkilä White 420W B2) in growing trays. Four control plants (tillers) per cultivar were planted into similar growing trays. Control plants kept at 4 °C were divided by planting timothy cultivars in one growing tray and other forage grasses and cereals in their own growing trays. The growing conditions in the greenhouse were 19/15 °C (day/night) at 16 h day length. During the winters the plants were irrigated by hand and fertilised (Kekkilä Watering Fertiliser N-P-K 17-4-25) weekly.

Plants were grown in the greenhouse for two weeks before the first viability observations. Thereafter, viability was observed once a week until no observable change in viability was noticed. For most plants this was 5–8 weeks after the first viability observations. Viability observations were done visually on a scale from one to five (1 dead; 5 fully viable). The time from planting to flowering (HD) was observed one to two times a week from control plants. The plants were considered to flower when at least one of the four control plants had produced a visible flower head. Experiments were terminated when no new flower heads

were observed to emerge. FLN of the main tiller was calculated at the end of experiment from the four control plants. The observations done from forage grasses and cereals were different between years (Table 3).

4.4 Freezing tolerance

The freezing tolerance of species was calculated as an LT_{50} -value (temperature which killed 50% of tillers) for the tested temperature series. Calculations were carried out using the values of the second observation when plants had been growing in the greenhouse for approximately three weeks and survival could be clearly evaluated. LT_{50} value for a cultivar was determined as the first temperature where 50% of the plants were dead and after which over 50% survival was not observed. Only three plants per temperature per cultivar were used for the freezing tolerance observations during winter 2009–2010 and for the observations in March in 2010–2011. In addition, viabilities of cultivars were pooled and the LT_{50} values of species were determined from that pooled data, which was done due to the lack of replicates within cultivars to calculate LT_{50} -values with reasonable reliability. Therefore, LT_{50} -values of cultivars are presented only in appendices (1–4).

5 Results

5.1 Weather and cold acclimation conditions during winters

5.1.1 Temperature, snow cover and precipitation

The lowest measured air temperatures varied between -22.8 and -26.1 °C in the four studied winters (Table 5). The number of freezing days when the ground was snow free was lowest in 2010–2011 and highest in 2009–2010. The end dates of the thermic growth periods were 28. September, 11. October, 8. November and 21. October between 2009–2013, respectively.

The duration of permanent snow cover varied between 88 and 147 days. In 2010–2011 permanent snow cover fell earlier than in the other studied years (Figure 1). The variation in the duration of the snow cover was mainly dependent on the date when the period of permanent snow cover started, not on the date of melting (Table 5.). In 2011–2012 the first snow cover fell three weeks later than in the second-latest year 2009–2010. On average the duration of the snow cover in Helsinki (1981–2010) lasts for 98 days (FMI 2012b). The insulating effect of a snow cover is substantial. In 2011–2012 daily mean topsoil temperature stayed at 0 °C (± 0.6 °C) during the whole snow covered period. Before and after the snow cover the top soil temperature fluctuated together with the ambient air temperature.

The precipitation sums for periods between the beginning of August and the end of November were 227 mm (2009), 163 mm (2010), 201 mm (2011) and 342 mm (2012). The precipitation sum in Kaisaniemi (Helsinki, Finland) in September 2012 was 160 mm which was the highest in the measured history and very high compared to the long-time average of 56 mm (1981–2010) (FMI 2012a).

Table 5. Describing factors for hardening conditions.

	2009– 2010	2010– 2011	2011– 2012	2012– 2013
Autumn and early winter				
Length of hardening period (5 °C as base temperature)	73	44	106	50
Beginning date of hardening period (5 °C as base temperature)	1.10.2009	12.10.2010	14.10.2011	12.10.2012
End date of hardening period*	13.12.2009	25.11.2010	28.1.2012	1.12.2012
CDD5 sum during hardening period (5 °C as base temperature)	103	59	141	60
FH-COLD (net CDD5 accumulation)	70	36	58	15
Precipitation sum during hardening period (mm)	127	99	285	76
Winter				
Winter period (days)	71	88	11	100
Number of freezing days	129	142	99	142
Number of freezing days during snow free period days**	38	15	22	16
Duration of permanent snow cover (days)	111	147	88	139
Minimum temperature (daily mean)	-18.7	-19.8	-19.2	-17.4
Minimum temperature (daily lowest)	-22.8	-24.0	-26.1	-23.3
W-RAIN (mean daily precipitation during WP) (mm)	1.2	2.1	1.3	1.9
Spring				
Spring frosts after the start of thermal growth period***	7	0	0	1

*Date of the first occurrence of <-10 °C daily minimum air temperature

** Calculated using daily lowest temperature and ground was considered snow free, when there was less than 5 cm of snow

*** After start of growing period (last day of first 5 day spell when daily mean air T > 5°C)

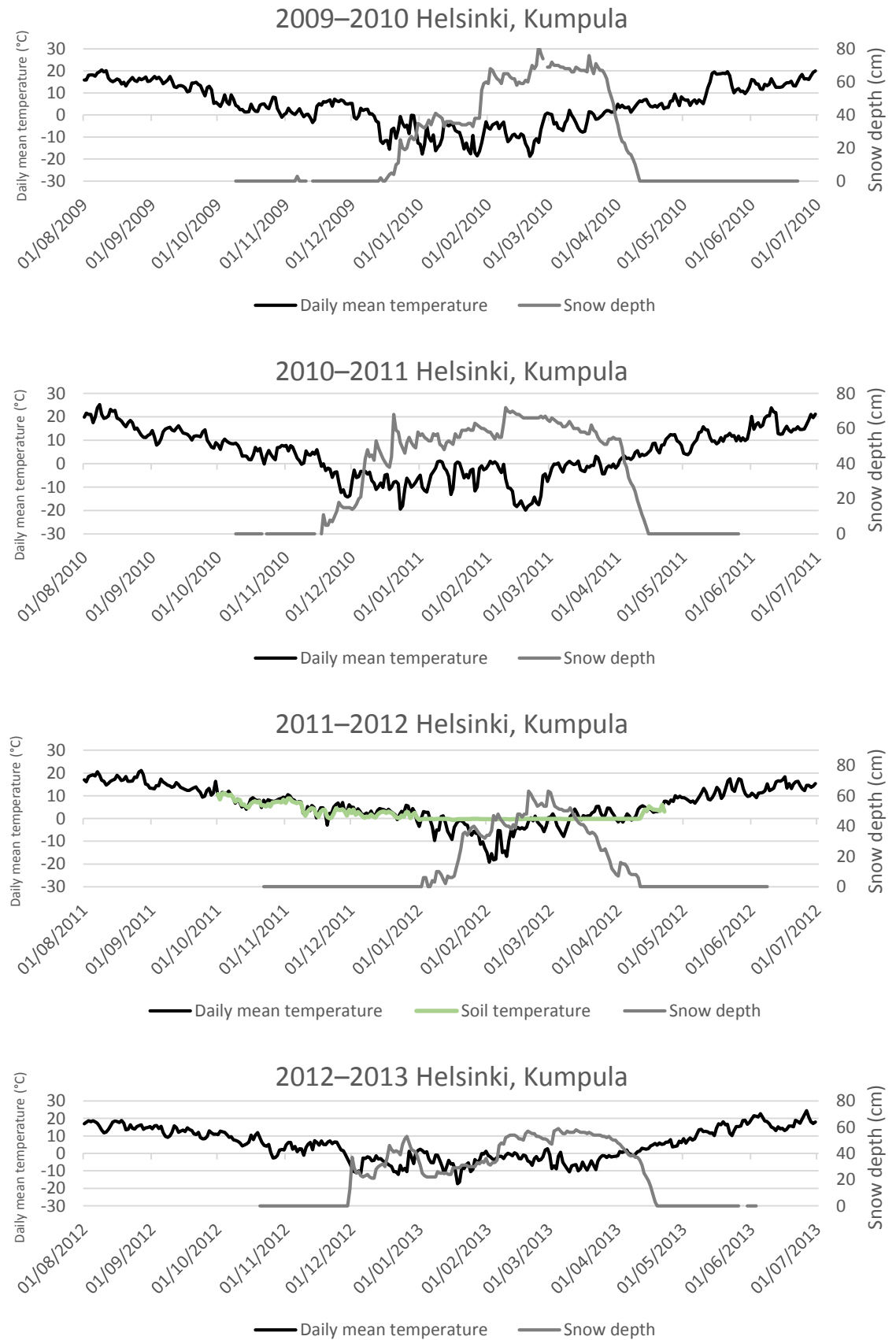


Figure 1. Daily mean air temperatures (black line) and snow depths (grey line) during study period 2009–2013. Daily mean top soil temperature (at the depth of 2 cm) is also presented for 2011–2012 (green line).

5.1.2 Hardening, winter and growth periods

The hardening period in 2011–2012 was the longest of the study period (Table 5.). It was over twice as long as the hardening period of winters 2010–2011 and 2012–2013. In 2011–2012 the cold degree day sum with 5°C as base temperature (CDD5 sum) was over twice as much as in 2010–2011 and 2012–2013. The winter period of winter 2011–2012 was substantially shorter than the winter periods of the other studied years. W-RAIN index was highest in 2010–2011 and lowest in 2009–2010.

In 2011 the CDD5 sum of the hardening period started to accumulate over a month later than in the other studied years (Figure 2). However, CDD5 accumulated quickly in 2011: CDD5 sum reached the level of years 2010 and 2012 in the beginning of December and at the end of the hardening period it ended as the highest. In 2009 the CDD5 sum accumulated earlier than in the other studied years and at the end of hardening period it reached the second highest amount after 2011. The hardening periods of 2010 and 2012 were quite similar in respect to CDD5 sum accumulation. Negative FH-COLD index describes the net accumulation of cold degree days during the hardening period. In 2011 the FH-COLD index remained negative until mid-December, while in 2009 and 2010 FH-COLD remained positive for the whole hardening period (Figure 3). In 2012 FH-COLD at the beginning of hardening period remained negative for the first two weeks but was positive at the beginning of November.

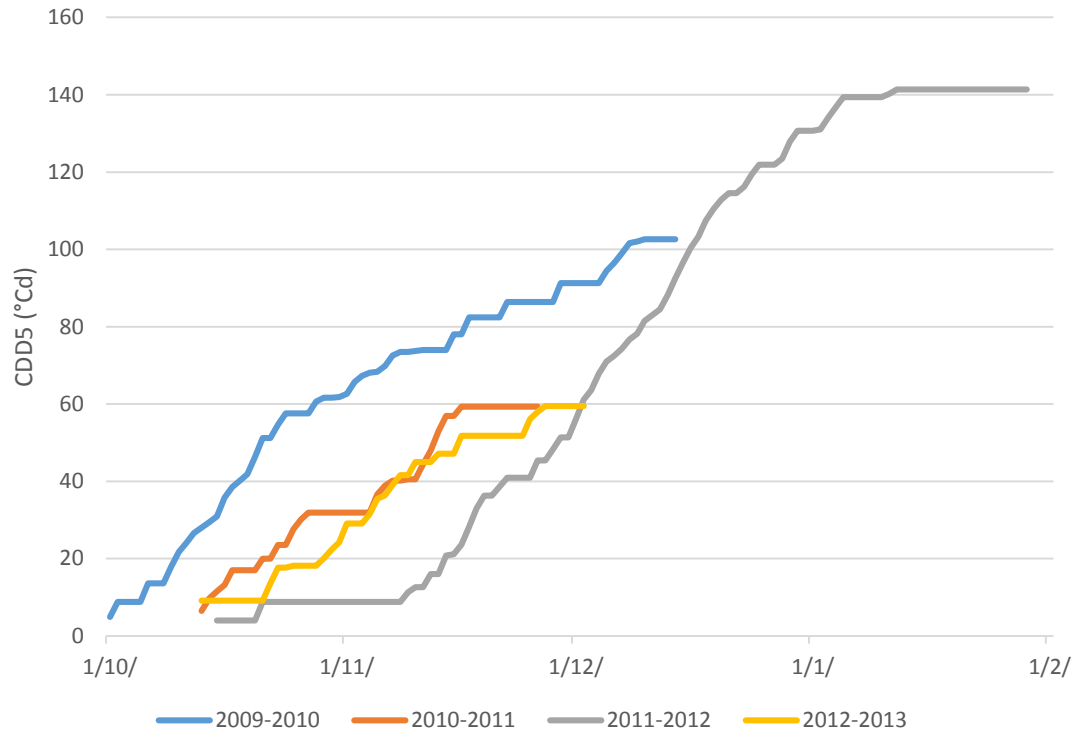


Figure 2. Development of CDD5 sums during hardening periods between years 2009-2013.

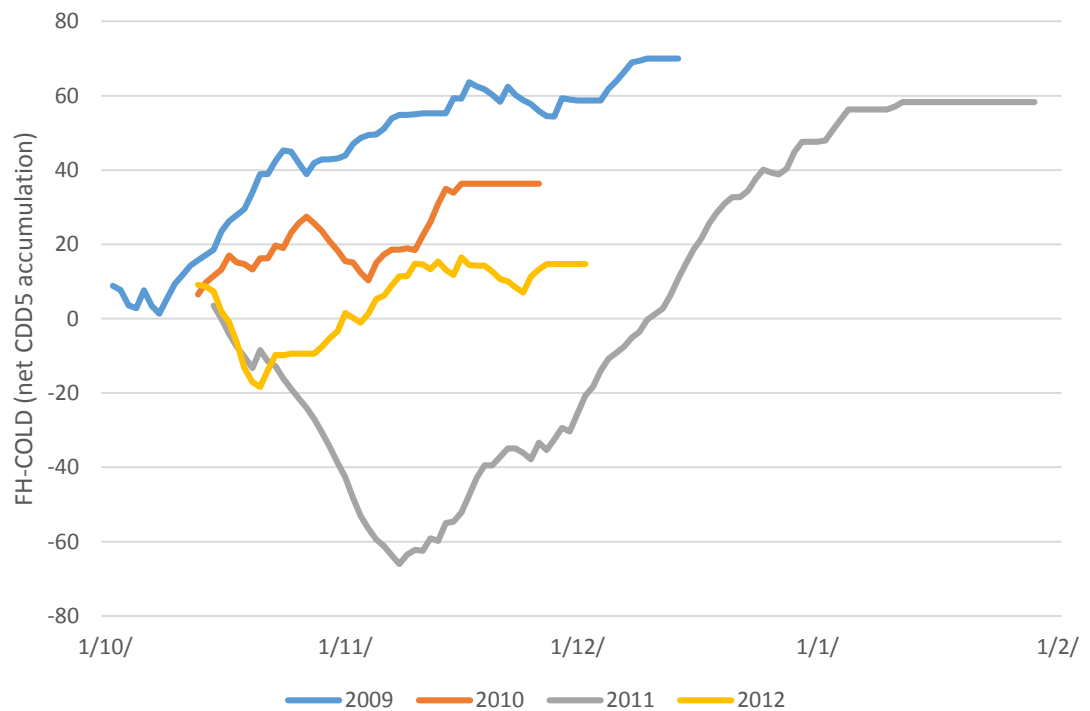


Figure 3. Development of FH-COLD indices during hardening periods between years 2009–2013.

5.2 Overwintering

5.2.1 Freezing tolerance

Timothy

Data for timothy were available for all four winters. In years 2009 and 2011 the minimum LT_{50} was measured in December-January, whereas in 2010 and 2012 the plants did not develop deeper freezing tolerance and a decrease in freezing tolerance was not observed (Figures 4–7). The minimum LT_{50} value at the beginning of the sampling period suggests that hardening happened before the first samples were taken from the field. The minimum freezing tolerances of different species varied significantly between the years. Timothy developed a minimum freezing tolerance of -12.5 and -15.0°C during the winters 2010–2011 and 2012–2013, whereas a minimum freezing tolerance in 2009–2010 and in 2011–2012 reached -22.5°C (Figure 6).

The highest LT_{50} values (lowest freezing tolerances) were -10.0°C during the first two winters and in 2010–2011 it was already reached at the third sampling date, 76 days after the start of the hardening period (Figure 6.). During winter 2011–2012, after reaching LT_{50} minimum, timothy dehardened linearly towards the last sampling in the beginning of April.

Meadow fescue and perennial ryegrass

LT_{50} data for meadow fescue were available for winters 2011–2012 and 2012–2013. In 2011 freezing tolerance decreased until the third sampling date and LT_{50} minimum (-17.5°C) was reached in mid-December (Figure 6). In 2012 the LT_{50} minimum (-12.5°C) was already reached at the first sampling date at the end of October (Figure 7).

Data for perennial ryegrass were available for the winters 2011–2012 and 2012–2013 and only for one cultivar. In 2011–2012 the LT_{50} value decreased in perennial ryegrass after the first sampling in October and reached its minimum ($-$

17.5°C) at the second sampling in the end of November (Appendix 3). Dehardening began after the end of December. In 2012 the lowest LT₅₀ value (-15°C) in perennial ryegrass was reached by the first sampling date at the end of October.

Winter cereals

Data for winter cereals were available for winters 2011–2012 and 2012–2013. In 2011 winter rye hardened before winter wheat and also had better freezing tolerance (lower LT₅₀ value) at the first sampling (Figure 6). In 2011 rye was sown almost three weeks earlier than wheat, which may have had an effect on the differences in hardening. In 2011–2012 rye reached LT₅₀ minimum at the first sampling (-17.5°C) and wheat at the second sampling (-17.5°C). However, onwards from the second sampling, wheat had better freezing tolerance than rye until the beginning of March. In 2012 winter rye and winter wheat reached LT₅₀ minimum (-20 and -10 °C, respectively) already at the first sampling date in the beginning of December. Thus, the decrease of LT₅₀ was observed only in the winter cereal data for 2011–2012. Winter cereals were killed towards the end of winter 2012–2013 and they had suffered extensive winter damage when the field was observed in the spring. In March 2013 the winter rye cultivar Riihi was the only winter cereal cultivar that had any surviving tillers at the highest temperatures in the freezing test.

Comparison of species

During the two winters (2011–2013) when freezing tolerance data for multiple species were available, timothy maintained its freezing tolerance best throughout the winter and developed better maximum freezing tolerance than meadow fescue and perennial ryegrass. During winter 2011–2012 meadow fescue reached its LT₅₀ minimum a month later than timothy. In 2012–2013, when deeper freezing tolerance was not reached, the forage grass species dehardened approximately at the same rate, although timothy had better freezing tolerance throughout the winter than meadow fescue and perennial ryegrass. In 2012 winter

rye had developed deeper freezing tolerance earlier than other species but dehardened rapidly after that.

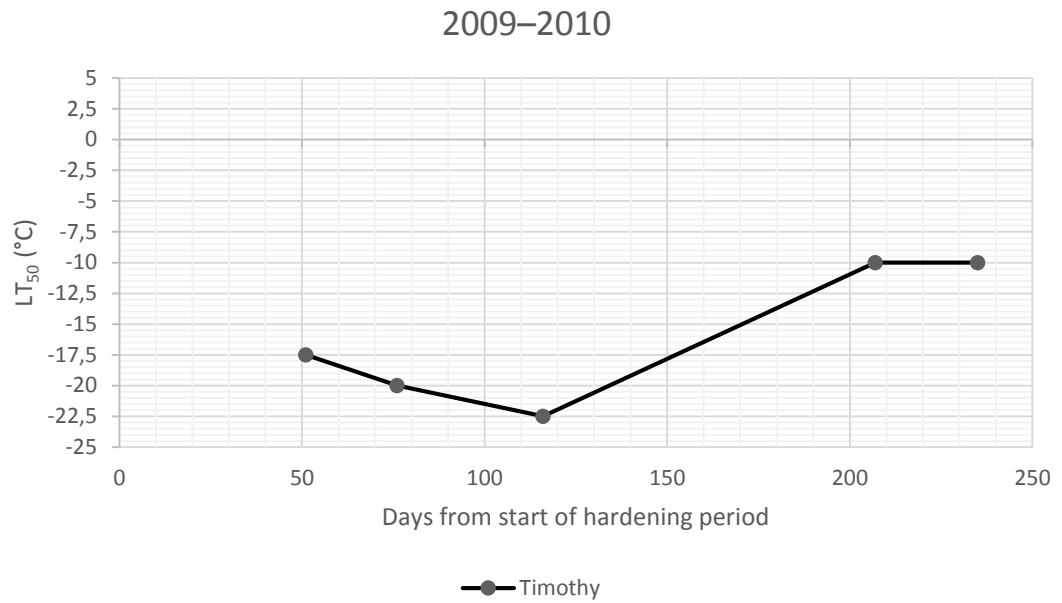


Figure 4. Freezing tolerance (LT₅₀) of timothy plants during winter 2009–2010. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=24. Start of the hardening period 1.10.2009.

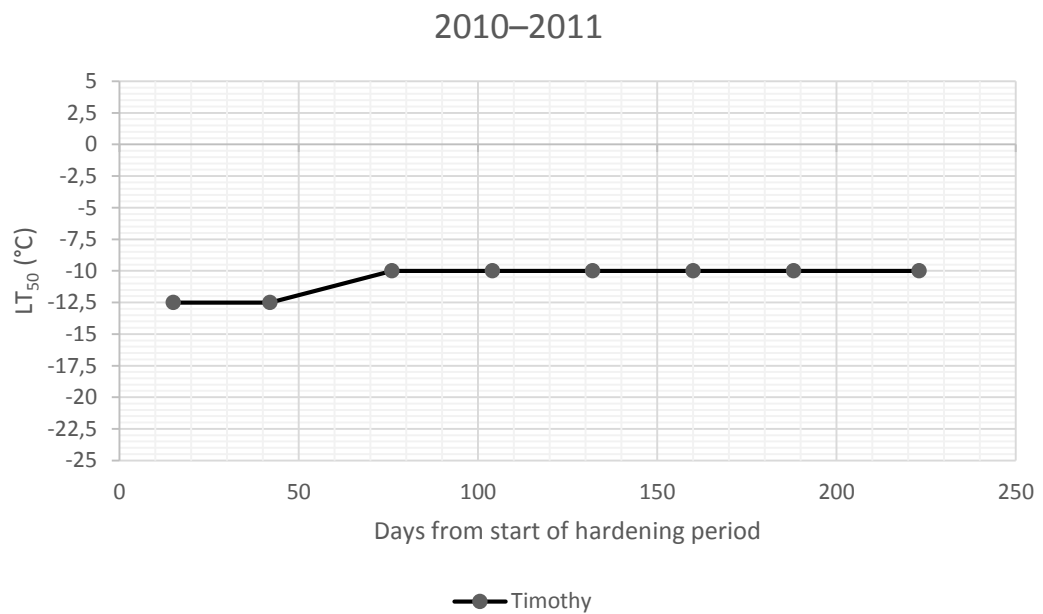


Figure 5. Freezing tolerance (LT₅₀) of timothy plants during winter 2010–2011. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=28. Start of the hardening period 12.10.2010.

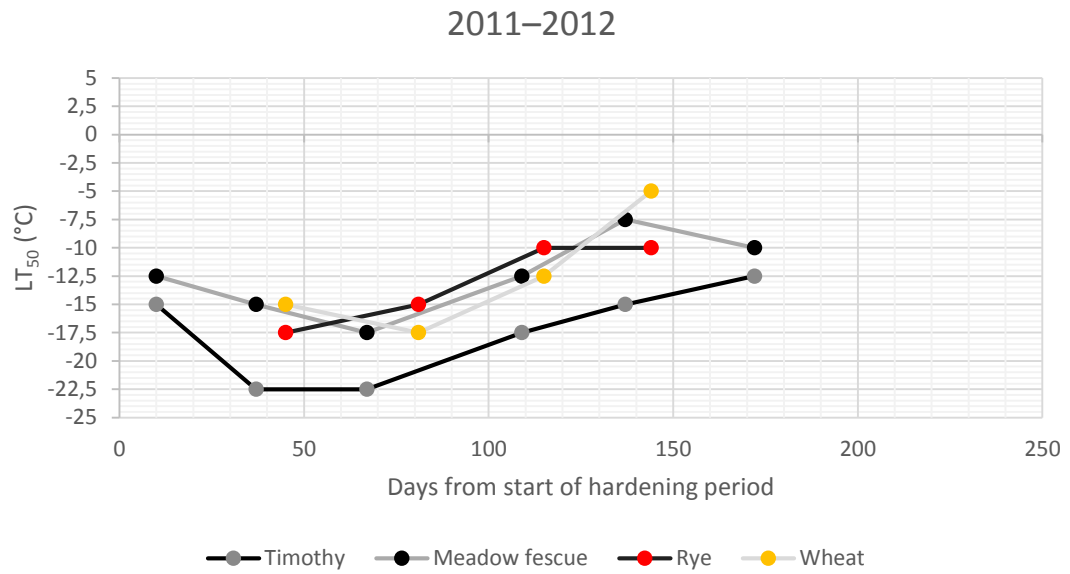


Figure 6. Freezing tolerance (LT_{50}) of timothy, meadow fescue, rye and wheat in 2011–2012. Number of plants used for calculation of LT_{50} for each temperature: timothy $n=24$, meadow fescue $n=12$, wheat $n=8$, rye $n=8$. Start of the hardening period 14.10.2011.

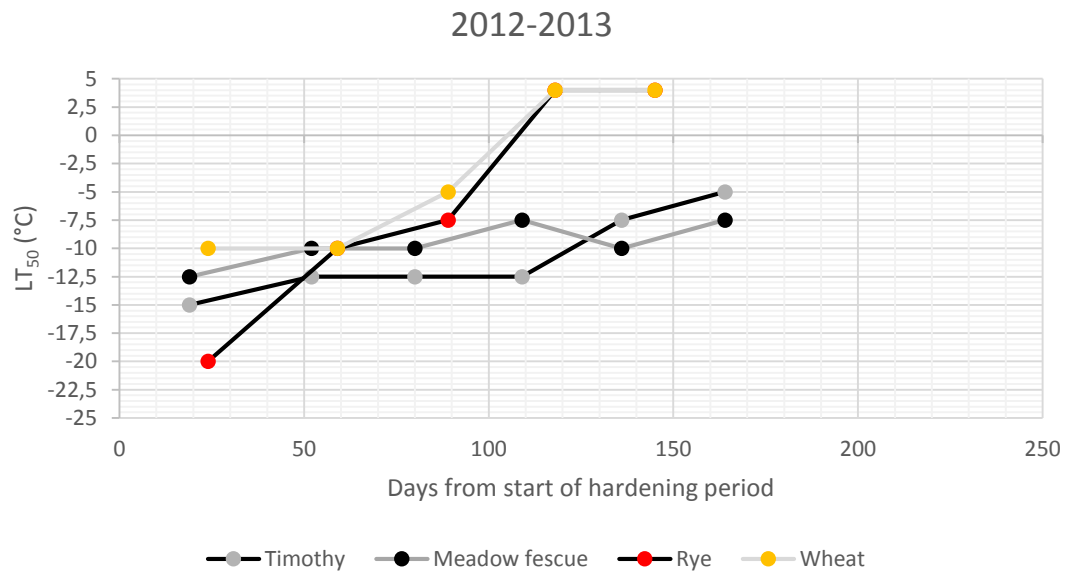


Figure 7. Freezing tolerance (LT_{50}) of timothy, meadow fescue, rye and wheat in 2012–2013. Number of plants used for calculation of LT_{50} for each temperature: timothy $n=24$, meadow fescue $n=12$, wheat $n=8$, rye $n=8$. Start of the hardening period 12.10.2012.

5.2.2 Heading date and final leaf number

The number of days between planting and flowering (HD) decreased in all studied species as the winter proceeded (Figures 8–11). Vernalisation fulfilment, measured as levelling off of the decrease in HD, occurred in all forage grass species between November and January.

Timothy

Differences in HD and FLN between the cultivars were largest during winter 2010–2011 (Figures 8 and 11), when the plants included were genetically the most diverse (Table 3). During winters 2011–2012 and 2012–2013 the HD curve levelled off during November and December, but in 2010–2011 the HD had large variation still at the end of January. In 2011–2012 all cultivars except Tuure started to flower at the first sampling in the end of October.

In timothy, the average FLNs differed within and between the years (Figure 11). The average FLN of sampling dates varied between five and ten leaves. In 2011 FLN was at its highest in the end of December and then dropped and levelled off during January. In timothy the FLN did not clearly reflect the development of vernalisation and HD, although a decrease in FLN was observed in 2012 at the same time as HD decreased between the second and third sampling dates.

Meadow fescue and perennial ryegrass

The vernalisation requirements, measured as decrease and levelling off of HD curve, in perennial ryegrass and meadow fescue were fulfilled before January during both of the studied winters 2011–2012 and 2012–2013 (Figure 9). The average FLNs of meadow fescue and perennial ryegrass varied from 3 to 5 and 4 to 6 leaves, respectively. In 2012 FLN did not correlate with HD. In 2011–2012 FLN values of meadow fescue decreased when HD also decreased between the second and third sampling dates (Figure 12), but in 2012–2013 no correlation between HD and FLN was observed, although HD decreased in all cultivars. A decrease in FLN was observed in perennial ryegrass during winter 2011–2012

but during winter 2012–2013 FLN increased after January. No correlation between the timing of decrease in FLN and HD was observed in perennial ryegrass.

Winter cereals

During winter 2011–2012, the samples collected at the first sampling in the end of November flowered, and the flowering time curve was thereafter levelled off (Figure 10). In 2012–2013 winter cereals had severe winter damage already in January and, therefore, data were available only for winter rye for February and only for one winter rye cultivar in March. Winter rye vernalised faster in 2012–2013 than winter wheat. FLN did not decrease significantly in winter cereals in 2012–2013 and it did not correlate with HD.

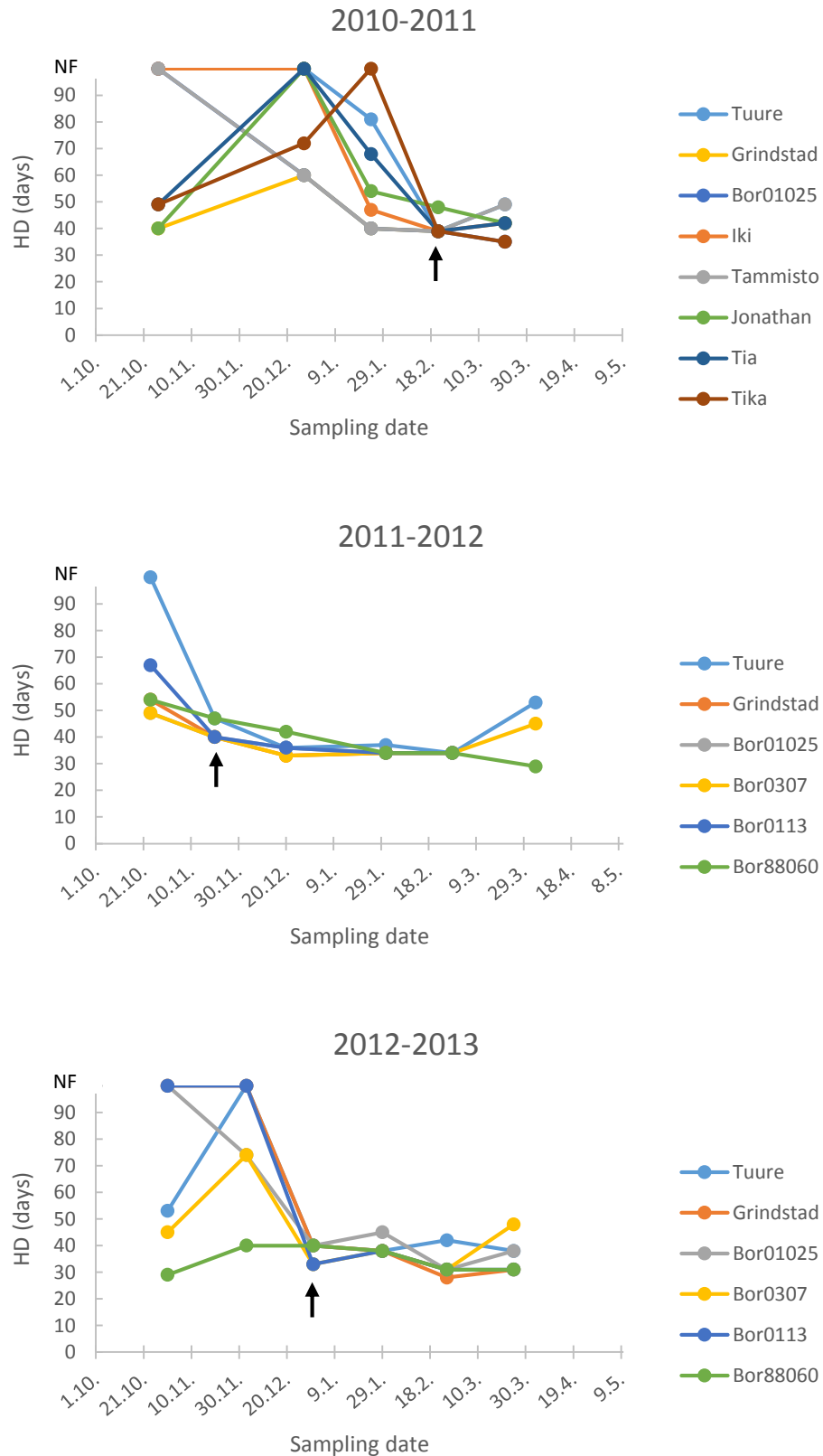


Figure 8. Progress of vernalisation measured as decrease in HD (heading date) during winter in timothy. Arrow indicates the point of vernalisation saturation. HD was measured from the first flowering control plant. NF = no flowering.

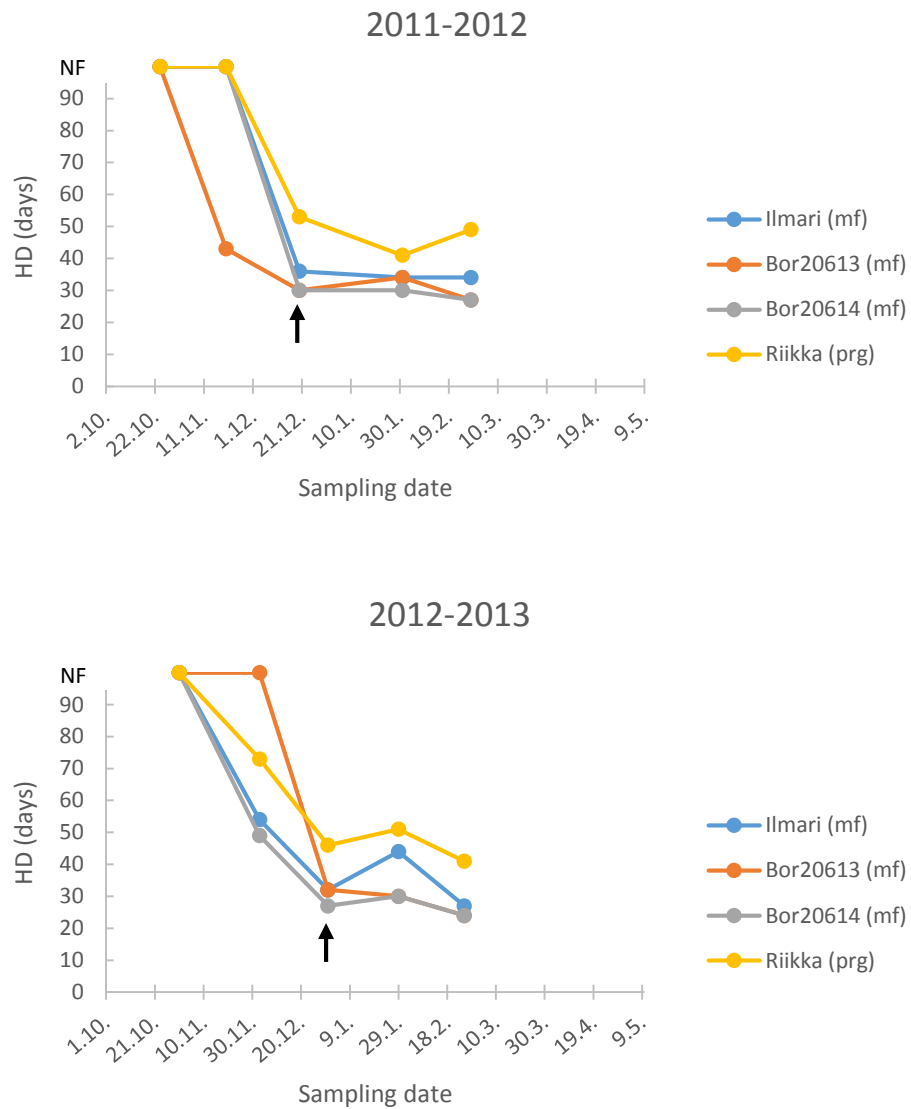


Figure 9.

Progress of vernalisation measured as decrease in HD (heading date) during winter in meadow fescue (mf) and perennial ryegrass (prg). Arrow indicates the point of vernalisation saturation. HD was measured from the first flowering control plant. NF = no flowering.

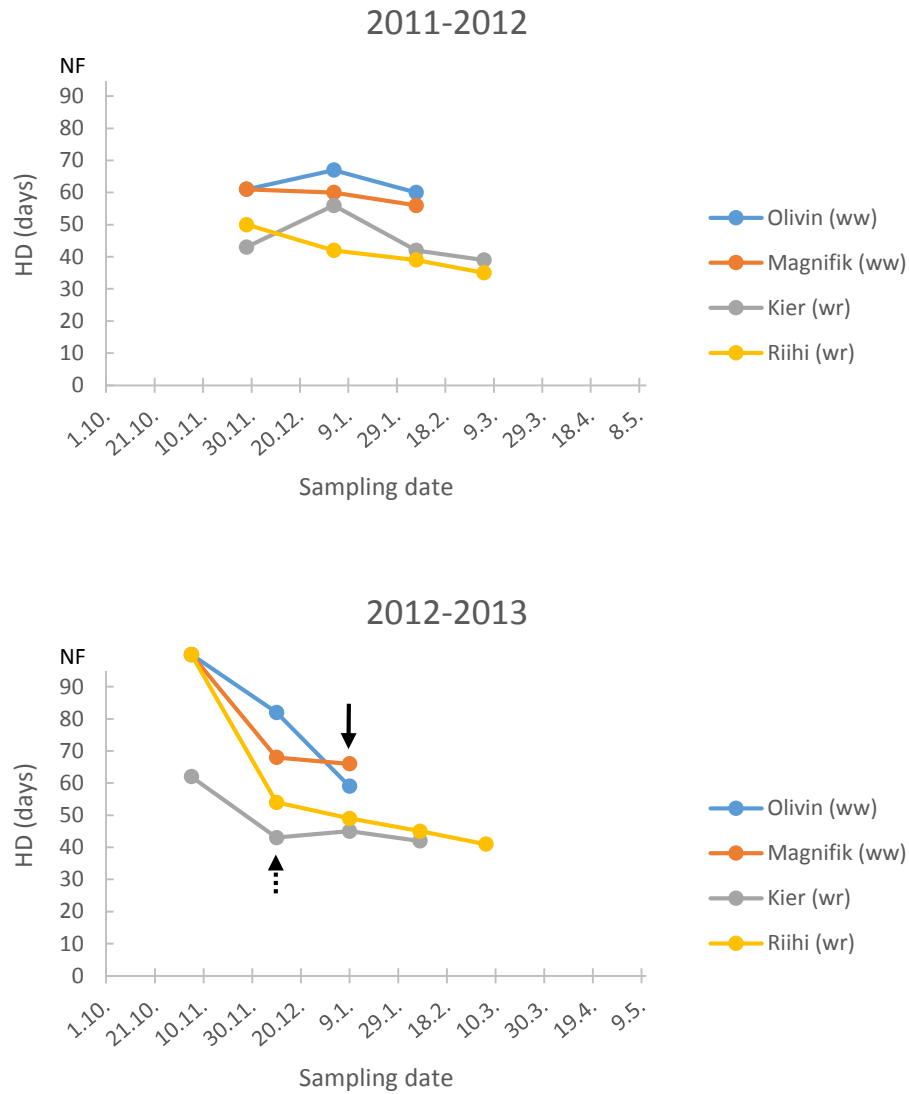


Figure 10.

Progress of vernalisation measured as decrease in HD (heading date) during winter in winter wheat (ww) and rye (wr). Arrow indicates the point of vernalisation saturation in rye (dashed arrow) and wheat (solid arrow). Fulfilment of vernalisation could not be determined for winter 2011–2012. HD was measured from the first flowering control plant. NF = no flowering.

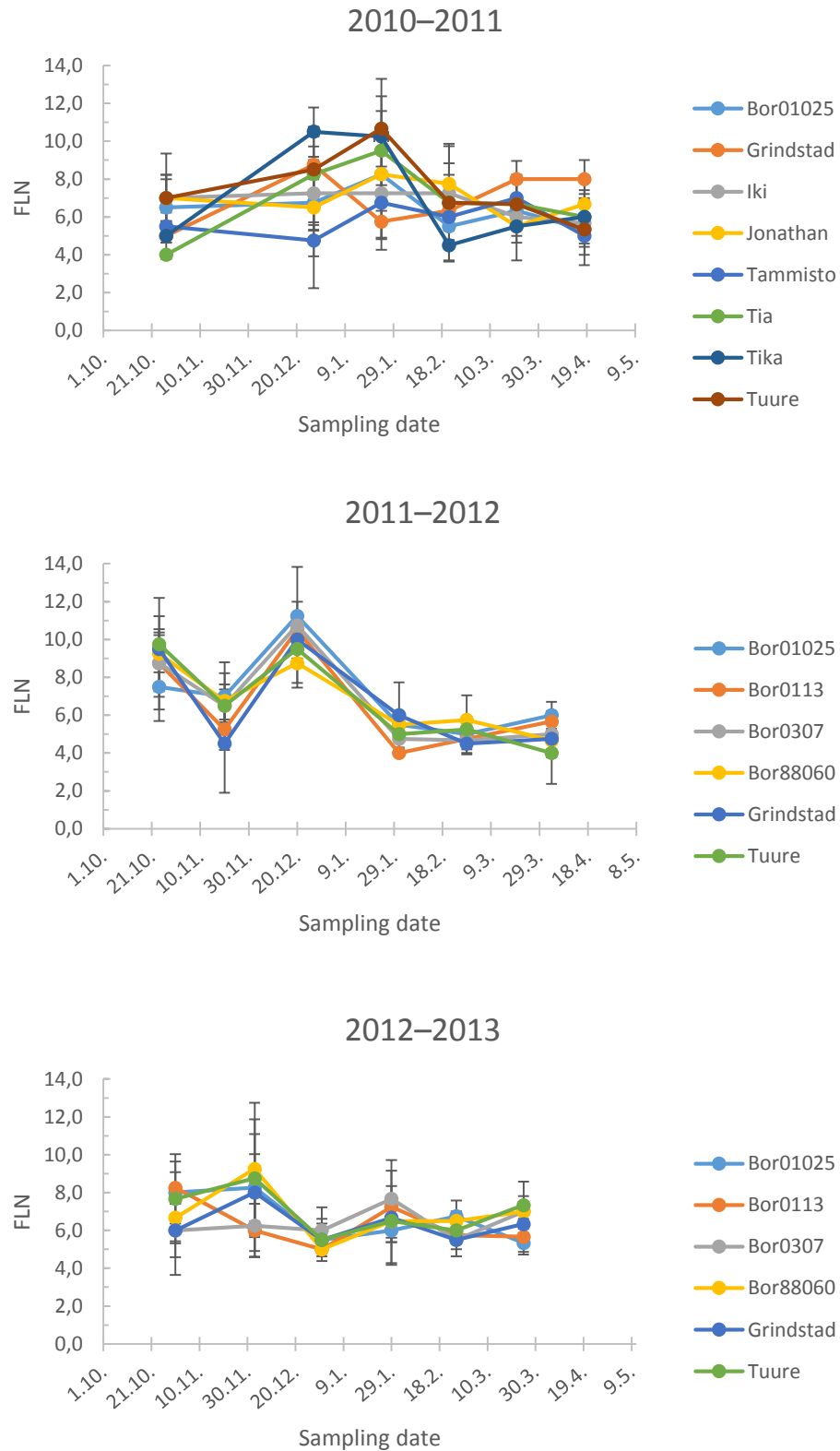


Figure 11. Development of FLN (final leaf number of main stem) during winter in timothy. FLN calculated as an average of living control plants, $n=1-4$. Vertical lines represent standard deviation.

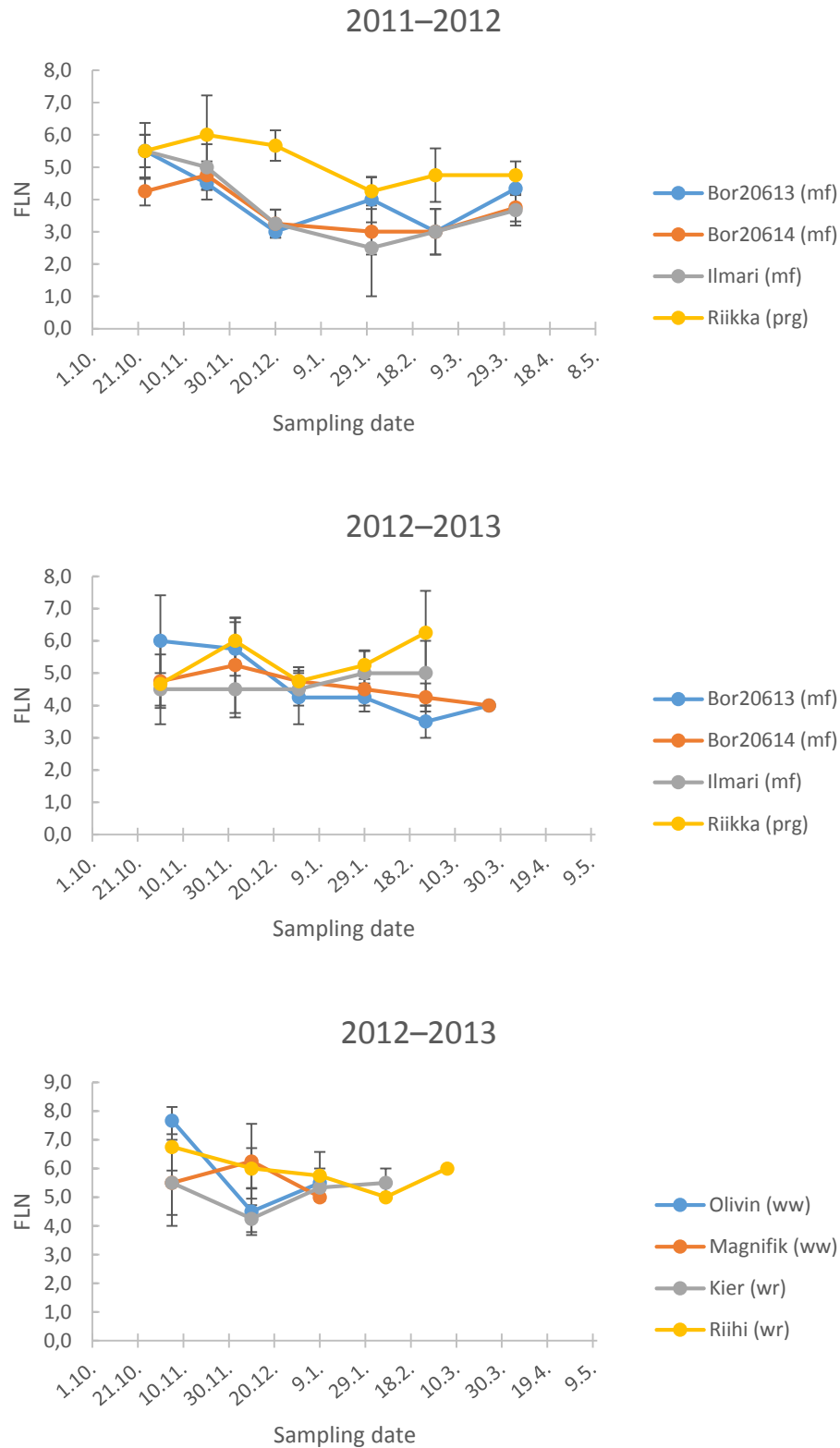


Figure 12. Development of FLN (final leaf number of main stem) during winter in meadow fescue (mf), perennial ryegrass (prg), winter wheat (ww) and winter rye (wr). FLN calculated as an average of living control plants, $n=1-4$. Vertical lines represent standard deviation.

6 Discussion

6.1 Freezing tolerance

The timing of cold acclimation and depth of freezing tolerance differed between species and differences in the minimum LT₅₀ values (maximum freezing tolerances) were large between years within species. The freezing tolerances recorded in this study were mostly in similar ranges as those recorded in other studies. For example in Holt (Norway) the LT₅₀ values reported for timothy have been -26.0 °C in 2005–2006 and 2006–2007 and in Særheim (Norway) -19.9 °C and -17.6 °C in 2005–2006 and 2006–2007, respectively (Höglind et al. 2008, Jørgensen et al. 2010). The LT₅₀ values for timothy did not differ much of those previously measured by Isolahti et al. (2004) (between -13.8 °C and -21.8 °C), although the plants in that experiment were hardened in controlled conditions. A larger difference was observed for LT₅₀ values measured in Viikki during winter 1993–1994, when LT₅₀-values were observed to be -9.9 °C for cultivar Bilbo and -12.2 °C for cultivar Iki (Kananen 1996). Nevertheless, it has to be kept in mind that LT₅₀ values measured in this study may not be fully comparable to the LT₅₀ values of other experiments due to the differences in cold treatments and different handling of plants prior to freezing experiments.

The differences in freezing tolerances between species were mostly as expected. Timothy had the lowest freezing tolerances of forage grasses and it maintained its freezing tolerance longer than other species. The maintenance of lower LT₅₀ in timothy towards the spring seemed to be a result of lower minimum freezing tolerance as the rate of dehardening did not vary much between species. Perennial ryegrass has been observed to lose its freezing tolerance earlier than timothy in other studies also, due to its lower initial frost tolerance (Jørgensen et al. 2010). In addition, the dehardening has been observed to be most rapid during the initial days when plants are exposed to dehardening conditions (Gay & Eagles 1991, Jørgensen et al. 2010). The relative dehardening rates of frost-tolerant cultivars have been reported to be lower or similar to those of less freezing-

tolerant cultivars (Jørgensen et al. 2010). Forage grasses respond dynamically to ambient temperature and, for example, perennial ryegrass can lose its freezing tolerance almost completely within 9 days of exposure to dehardening conditions (Jørgensen et al. 2010). In this study, the amount of precipitation during the autumn did not seem have a substantial effect on the development of freezing tolerance, since low freezing tolerances were measured both after a dry and a wet autumn.

In conclusion, all forage grass species hardened well enough during all winters to withstand the winter stresses and no severe winter damage was observed during the winters in the studied species. This was despite the deeper freezing tolerance having developed in the forage grasses during only two winters. Thus, the snow cover probably protected plants from winter damages during the two winters when the freezing tolerances were lower. Winter cereals experienced severe winter damage during the last winter (2012–2013) and in the autumn before the damage the plants were also exposed to higher than normal amounts of precipitation (FMI 2012a). Jørgensen et al. (2010) discussed that in timothy a high level of frost tolerance could prevent dehardening during the winter due to a longer time that is required for plant to lose its freezing tolerance. In this study, winter rye lost its freezing tolerance quickly, even though it had the best initial freezing tolerance in 2012–2013.

6.2 Vernalisation

Meadow fescue, perennial ryegrass and winter cereals require vernalisation before flowering initiates (Chouard 1960, Heide 1994, Fowler et al. 1999). The HD of meadow fescue and perennial ryegrass decreased as predicted during both winters when they were studied (2011–2012 and 2012–2013). In winter cereals HD decreased as predicted in 2012, but in 2011 the decrease was not observed, probably because vernalisation could have happened before the first sampling. The HD in timothy also decreased during all three observed winters (2010–2013) as was expected from earlier studies by Seppänen et al. 2010. The FLN varied between sampling dates and no clear relationship between FLN and

HD was observed. Thus, in this study FLN could not be used as an estimate for the development of vernalisation during the vernalisation period. Even winter wheat and winter rye did not show a significant correlation between FLN and HD, although it was expected based on studies by Fowler et al. 1996 and Mahfoozi et al. 2001. In studies with winter wheat FLN has decreased and levelled off after approximately 35 days of acclimation in 4 °C (Mahfoozi et al. 2001).

The fulfilment of vernalisation requirement occurred in December and January in all forage grass species during each of the years. Thus, the longer hardening periods did not seem to have an effect on the timing of vernalisation fulfilment. Fowler et al. (1996) have reported that vernalised plants are more prone to dehardening than non-vernalised plants. In our study no differences were detected in the dehardening of forage grasses after differing winters. However, in 2012–2013 winter rye had a good freezing tolerance prior to the winter damage and quick dehardening in December.

6.3 Comparison of freezing tolerance to conditions during hardening and overwintering

Annual variation exists in the duration of snow covered periods and number of freezing days. During the studied period between 2009 and 2013, the shortest snow covered period was 88 days during winter 2011–2012, which is still more than 46 days of snow cover predicted for 2050 for southern Finland (Höglind et al. 2013). The number of freezing days was already lower in 2011–2012 (99 days) than that predicted for southern Finland by 2055 (100 days) (Peltonen-Sainio et al. 2009). The lowest winter temperatures always occurred when thick snow cover was present, and thus plants were not exposed to these potentially the most lethal temperatures. The warm period in December 2011 lasted for 30 days, which was the longest period between December and February when temperature remained above 0 °C since 1961 (FMI 2012a).

The onset of cold acclimation was not observed during the data collection periods due to the first samplings being taken too late, at the end of October or during

November. However, the development of deeper freezing tolerance in all species was detected during the winters with long hardening periods.

Warm temperatures which led to negative FH-COLD indices at the beginning of the hardening period in 2011–2012 did not seem to prevent the development of a good freezing tolerance in the studied species. The plants developed the deepest freezing tolerances during winters 2009–2010 and 2011–2012 when the FH-COLD index was the highest and thus, when the highest amount of hardening temperatures was present. In addition, the hardening periods were longest and the durations of permanent snow covers shortest during the two winters during which the best freezing tolerances were achieved. In winters 2009–2010 and 2011–2012 the number of freezing days when the snow cover was not present was the highest, which may also have had an effect on the development of freezing tolerance.

During all of the studied winters the first samples were collected after the calculated beginning date of the hardening period. Hardening had already been taking place for 10–52 days before the first sampling dates during the four observed winters, based on the calculated beginning dates of hardening periods. This was probably the reason why no decreases were observed in LT_{50} -values during winters 2010–2011 and 2012–2013. In studies made with winter wheat, LT_{50} decreased to minimum approximately after 35 days of acclimation in 4 °C (Mahfoozi et al. 2001).

7 Conclusions

The development of freezing tolerance, FLN and HD of the studied species were compared with different hardening and winter period factors. A correlation between long hardening periods (HP) and a good freezing tolerance (LT_{50}) was found. During the long hardening periods also the temperature sums of cold acclimation inducing temperatures (FH-COLD) accumulated the most. Even the most winter-tolerant species timothy did not develop a good freezing tolerance during the two short hardening periods. During these two winters the snow cover

probably protected plants from winter damage. Species differed in their freezing tolerances, timothy having better freezing tolerance during both of the winters than meadow fescue, perennial ryegrass and winter cereals.

Overall, a correlation between the timing of vernalisation (decrease in HD) and freezing tolerance (LT_{50}) was not found in 2010–2011 and 2012–2013, but the development of HD showed some correlation with good hardening in 2011–2012. However, the timing of vernalisation fulfilment was only roughly estimated in this study and further processing of the data is required to find out more accurately the timing of vernalisation saturation points.

The timing of hardening and vernalisation processes were determined during the various hardening periods and this information can be used in the planning of future research projects. In this study, freezing tolerances showed that the hardening processes started already in October and therefore the first sampling should be done already in August to get more accurate data of the beginning of cold acclimation processes in field conditions.

Overall there is a lack of good data of the development of freezing tolerances in field conditions of forage grasses grown in northern countries. Only a small number of studies have been conducted to measure the dynamics of hardening processes in field conditions with current modern cultivars used in Finland. Even fewer studies have been made on cultivars with clearly differing traits that might be expected to be relevant in future conditions. Thus, well prepared freezing-tolerance experiments over multiple years including genotypes of clear southern and northern origin would give more information on the genotypic variation in these traits within species and reveal the breeding potential in important traits for adaptation.

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References

Andrews, C., 1987. Low-temperature stress in field and forage crop production-an overview. *Canadian Journal of Plant Science*, 67(4), pp. 1121-1133.

Andrews, C. and Pomeroy, M.K., 1989. Physiological properties of plants affecting ice-encasement tolerance. *Ice. Agric. Sci*, 2, pp. 41-51.

Baker, C. K. and Gallagher J. N., 1983. The development of winter wheat in the field. 2. The control of primordium initiation rate by temperature and photoperiod. *The Journal of Agricultural Science*, 101, pp 337-344.

Bélanger, G., Rochette, P., Castonguay, Y., Bootsma, A., Mongrain, D. and Ryan, D.A., 2002. Climate change and winter survival of perennial forage crops in eastern Canada. *Agronomy Journal*, 94(5), pp. 1120-1130.

Bergjord, A.K., Bonesmo, H. and Skjelvåg, A.O., 2008. Modelling the course of frost tolerance in winter wheat: I. Model development. *European Journal of Agronomy*, 28(3), pp. 321-330.

Bertrand, A. and Castonguay, Y., 2003a. Plants adaptations to overwintering stresses and implications of climate change. *Can. J. Bot.*, 81, pp. 1145-1152.

Bertrand, A., Castonguay, Y., Nadeau, P., Laberge, S., Michaud, R., Bélanger, G. and Rochette, P., 2003b. Oxygen deficiency affects carbohydrate reserves in overwintering forage crops. *Journal of experimental botany*, 54(388), pp. 1721-1730.

Bond, D., Dennis, E. and Finnegan E., 2011. The low temperature response pathways for cold acclimation and vernalization are independent. *Plant, Cell and Environment*, 34, pp. 1737-1748.

Carter, T., 1998. Changes in the thermal growing season in Nordic countries during the past century and prospects for the future. *Agricultural and Food Science in Finland* 7, pp. 161–179.

Chouard, P., 1960. Vernalisation and its relation to dormancy. *Annu. Rev. Plant Physiol.*, 11, pp.191–238.

Finnish Meteorological Institute. 2012. Monthly weather statistics. Cited on 10.4.2014. Published on 1.11.2013. Available online:
<http://ilmatieteenlaitos.fi/syyskuu>

Finnish Meteorological Institute. 2012b. Winter statistics. Cited on 15.4.2014. Published on 22.2.2012. Available online: <http://ilmatieteenlaitos.fi/lumitilastot>

Fowler, D., Gusta, L. and Tyler, N., 1981. Selection for winterhardiness in wheat. III. Screening methods. *Crop Science*, 21(6), pp. 896-901.

Fowler, D., Limin, A., Wang, S. and Ward, R., 1996. Relationship between low-temperature tolerance and vernalization response in wheat and rye. *Can. J. Plant Sci.* 76, pp. 37-42.

Fowler, D., Limin, A. and Ritchie, J., 1999. Low-temperature tolerance in cereals: model and genetic interpretation. *Crop Science*, 39(3), pp. 626-633.

Gay A. and Eagles. C., 1991. Quantitative analysis of cold hardening and dehardening in *Lolium*. *Annals of Botany*, 67(4), pp. 339-345.

Griffith, M., Lumb C., Wiseman S.B., Wisniewski M., Johnson R.W. and Marangoni, A.G., 2005. Antifreeze proteins modify the freezing process in planta. *Plant Physiol.* 138, pp. 330–340.

Gusta, L.V., Wisniewski, M., Nesbitt, N.T. and Tanino, K.T. 2003. Factors to consider in artificial freeze tests. *Acta Hort.*, 618, pp. 493-507

Hakala, K. & Mela, T. 1996. The effects of prolonged exposure to elevated temperatures and elevated CO₂ levels on the growth, yield and dry matter partitioning of field sown meadow fescue (*Festuca pratensis*, cv. Kalevi). *Agricultural and Food Science in Finland* 5, pp. 285–298.

Heide, O., 1994. Control of flowering and reproduction in temperate grasses. *New Phytologist*, 128(2), pp. 347-362.

Hisano, H., Kanazawa, A., Kawakami, A., Yoshida, M., Shimamoto, Y. and Yamada, T., 2004. Transgenic perennial ryegrass plants expressing wheat fructosyltransferase genes accumulate increased amounts of fructan and acquire increased tolerance on a cellular level to freezing. *Plant Sci.* 167, pp. 861–868.

Hofgaard, I., Vollsnes, A., Marum, P., Larsen, A. and Tronsmo, A., 2003. Variation in resistance to different winter stress factors within a full-sib family of perennial ryegrass. *Euphytica*, 134(1), pp. 61-75.

Hulke, B., Watkins, E., Wyse, D. and Ehlke, N., 2008. Freezing tolerance of selected perennial ryegrass (*Lolium perenne* L.) accessions and its association with field winterhardiness and turf traits. *Euphytica*, 163(1), pp. 131-141.

Huner, N., Öquist, G., Hurry, V., Krol, M., Falk, S. and Griffith, M., 1993. Photosynthesis, photoinhibition and low temperature acclimation in cold tolerant plants. *Photosynthesis Research*, 37(1), pp. 19-39.

Höglind, M., Jørgensen, M., Østrem, L., Bakken, A. and Thorsen, S., 2008. Overwintering of timothy and perennial ryegrass in Norway from a climate change perspective, *Proceedings of 22nd general meeting of the European Grassland Federation, Uppsala (Sweden). Grassland Science in Europe* 13 2008, pp. 203-205.

Höglind, M., Hanslin, H. and Mortensen, L., 2011. Photosynthesis of *Lolium perenne* L. at low temperatures under low irradiances. *Environmental and experimental botany*, 70(2), pp. 297-304.

Höglind, M., Thorsen, S. and Semenov, M., 2013. Assessing uncertainties in impact of climate change on grass production in Northern Europe using ensembles of global climate models. *Agricultural and Forest Meteorology*, 170, pp. 103-113.

Höglind, M., Bakken, A., Jørgensen, M. and Østrem, L., 2010. Tolerance to frost and ice encasement in cultivars of timothy and perennial ryegrass during winter. *Grass and Forage Science*, 65(4), pp. 431-445.

IPCC, 2007: *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L. Miller (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

IPCC, 2013: *Summary for Policymakers. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Isolahti, M., Nissinen, O., Lüscher, A., Jeangros, B., Kessler, W., Huguenin, O., Lobsiger, M., Millar, N. and Suter, D., 2004. Role of storage carbohydrates in hardening and resistance of timothy genotypes to frost and *Typhula* spp. Land use systems in grassland dominated regions: Proceedings of the 20th General Meeting of the European Grassland Federation, Luzern, Switzerland, 21-24 June 2004. 2004, Edited by A. Lüscher et al., *Grassland science in Europe* 9: pp. 434-436.

Isolahti, M. 2010. Timotein maantieteelliset geenipoolit ja niiden käyttö jalostusohjelmissa. Suomen Maataloustieteellisen Seuran julkaisu no 26. Edited by A. Hopponen. Cited on 1.2.2014. Published on 11.1.2010. Available online: <http://www.smts.fi/jul2010/esite2010/049.pdf> ISBN 978-951-9041-54-4.

Jylhä, K., Tuomenvirta, H. & Ruosteenoja, K. 2004. Climate change projections for Finland during the 21st century. *Boreal Environment Research* 9: 127–152.

Jørgensen, M., Østrem, L. and Höglind, M., 2010. De-hardening in contrasting cultivars of timothy and perennial ryegrass during winter and spring. *Grass and Forage Science*, 65(1), pp. 38-48.

Kalberer, S., Wisniewski, M. and Arora, R., 2006. Deacclimation and reacclimation of cold-hardy plants: current understanding and emerging concepts. *Plant Science*, 171(1), pp. 3-16.

Kalberer, S., Arora, R., Leyva-Estrada, N. and Krebs, S., 2007. Cold hardiness of floral buds of deciduous azaleas: Dehardening, rehardening, and endodormancy in late winter. *Journal of the American Society for Horticultural Science*, 132(1), pp. 73-79.

Kananen, M. 1996. Master's thesis. Sokerit, proteiinit ja proliini timotein (*Phleum pratense*) kylmäkaraistumisen kuvaajina. University of Helsinki, Finland. 85 pp.

Kangas A. and Harmoinen T. 2012a. Peltokasvilajikkeet 2012. ProAgria Keskusten Liitto. 96 pp.

Kangas A., Högnäsbacka M., Kujala M., Laine A., Niskanen M., Jauhiainen L. and Nikander H., 2012b. Results of official variety trials 2005-2012. MTT Agrifood Research Finland. Cited 5.11.2013. Available online: <http://www.mtt.fi/mttraportti/pdf/mttraportti75.pdf>

Klein Tank, A. and Wijngaard, J., et al., 2002. Daily dataset of 20th-century surface air temperature and precipitation series for the European Climate Assessment. *International Journal of Climatology* 22. pp. 1441–1453.

Klein Tank, A. and Können, G. 2003. Trends in Indices of Daily Temperature and Precipitation extremes in Europe, 1946-99. *J. Climate*, 16, pp. 3665–3680.

Krause G.H. 1988. Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms. *Physiol. Plant.* 74, pp. 566–574.

Larsen, A., 1978. Freezing tolerance in grasses. *Meld Norg. Landbr. Høghsk.* 57, 56 pp.

Livingston, D., Hinch, D. and Heyer, A., 2009. Fructan and its relationship to abiotic stress tolerance in plants. *Cell. Mol. Life Sci.* 66, pp. 2007–2023.

Maa- ja metsätalousministeriön tietopalvelukeskus TIKE. 2013. Utilized agricultural area in 2012. Cited on 10.9.2013
http://www.maataloustilastot.fi/sites/default/modules/pubdlnet/pubdlnet.php?file=http://www.maataloustilastot.fi/sites/default/files/kaytossa_olevan_maatalousmaa_2012_8.1.2013.xls&nid=2883

Mahfoozi, S., Limin, A. & Fowler, D., 2001. Influence of Vernalization and Photoperiod Responses on Cold Hardiness in Winter Cereals. *Crop Sci.*, 41, pp. 1006–1011.

Olesen, J. and Bindi, M., 2002. Consequences of climate change for European agricultural productivity, land use and policy. *European Journal of Agronomy*, 16, pp. 239–262.

Olesen, J.E., Trnka, M., Kersebaum, K., Skjelvåg, A., Seguin, B., Peltonen-Sainio, P., Rossi, F., Kozyra, J. and Micale, F., 2011. Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy*, 34(2), pp. 96–112.

Pearce, R.S., 2001. Plant freezing and damage. *Ann. Bot.*, 87, pp. 417–424.

Peltonen-Sainio, P., Jauhiainen, L., Hakala, K. and Ojanen, H. 2009. Climate change and prolongation of growing season: changes in regional potential for field crop production in Finland. *Agricultural and Food Science*, 18, pp. 171-190.

Peltonen-Sainio, P., Jauhiainen, L. and Hakala, K. 2011. Crop responses to temperature and precipitation according to long-term multi-location trials at high-latitude conditions. *Journal of Agricultural Science*, 149, pp. 49-62.

Pollock, C. and Cairns, A., 1991. Fructan metabolism in grasses and cereals. *Annual review of plant biology*, 42(1), pp. 77-101.

Rognli, O., 2013. Breeding for Improved Winter Survival in Forage Grasses. *Plant and Microbe Adaptations to Cold in a Changing World*. Springer, pp. 197-208.

Ruosteenoja, K., H. Tuomenvirta, and K. Jylha, 2007: GCM-based regional temperature and precipitation change estimates for Europe under four SRES scenarios applying a super-ensemble pattern-scaling method. *Clim. Change*, 81, pp. 193-208.

Ruosteenoja, K., Räisänen, J. and Pirinen, P., 2011. Projected changes in thermal seasons and the growing season in Finland. *Int. J. Climatol.*, 31, pp. 1473–1487.

Sakai, A., Larcher, W. 1987. Frost survival of plants: responses and adaptation to freezing stress. Berlin, pp. 321.

Sandve, S.R., Kosmala, A., Rudi, H., Fjellheim, S., Rapacz, M., Yamada, T. and Rognli, O.A., 2011. Molecular mechanisms underlying frost tolerance in perennial grasses adapted to cold climates. *Plant Science*, 180(1), pp. 69-77.

Seppänen, M.M., Pakarinen, K., Jokela, V., Andersen, J.R., Fiil, A., Santanen, A. and Virkajärvi, P., 2010. Vernalisation response of *Phleum pratense* and its relationships to stem lignification and floral transition. *Annals of botany*, 106(5), pp. 697-707.

Skinner, H., 2007. Winter carbon dioxide fluxes in humid-temperate pastures. *Agricultural and Forest Meteorology*, 144(1), pp. 32-43.

Thorsen, S.M. and Höglind, M., 2010a. Modelling cold hardening and dehardening in timothy. Sensitivity analysis and Bayesian model comparison. *Agricultural and Forest Meteorology*, 150(12), pp. 1529-1542.

Thorsen, S.M. and Höglind, M., 2010b. Assessing winter survival of forage grasses in Norway under future climate scenarios by simulating potential frost tolerance in combination with simple agroclimatic indices. *Agricultural and Forest Meteorology*, 150(9), pp. 1272-1282.

Thorsen, S.M., Roer, A. and Van Oijen, M., 2010. Modelling the dynamics of snow cover, soil frost and surface ice in Norwegian grasslands. *Polar Research*, 29(1), pp. 110-126.

Trnka, M., 2011. Agroclimatic conditions in Europe under climate change. *Global Change Biology*, 17, pp. 2298–2318.

Vijn, I. and Smeekens, S., 1999. Fructan: more than a reserve carbohydrate? *Plant physiology*, 120, pp. 351-359.

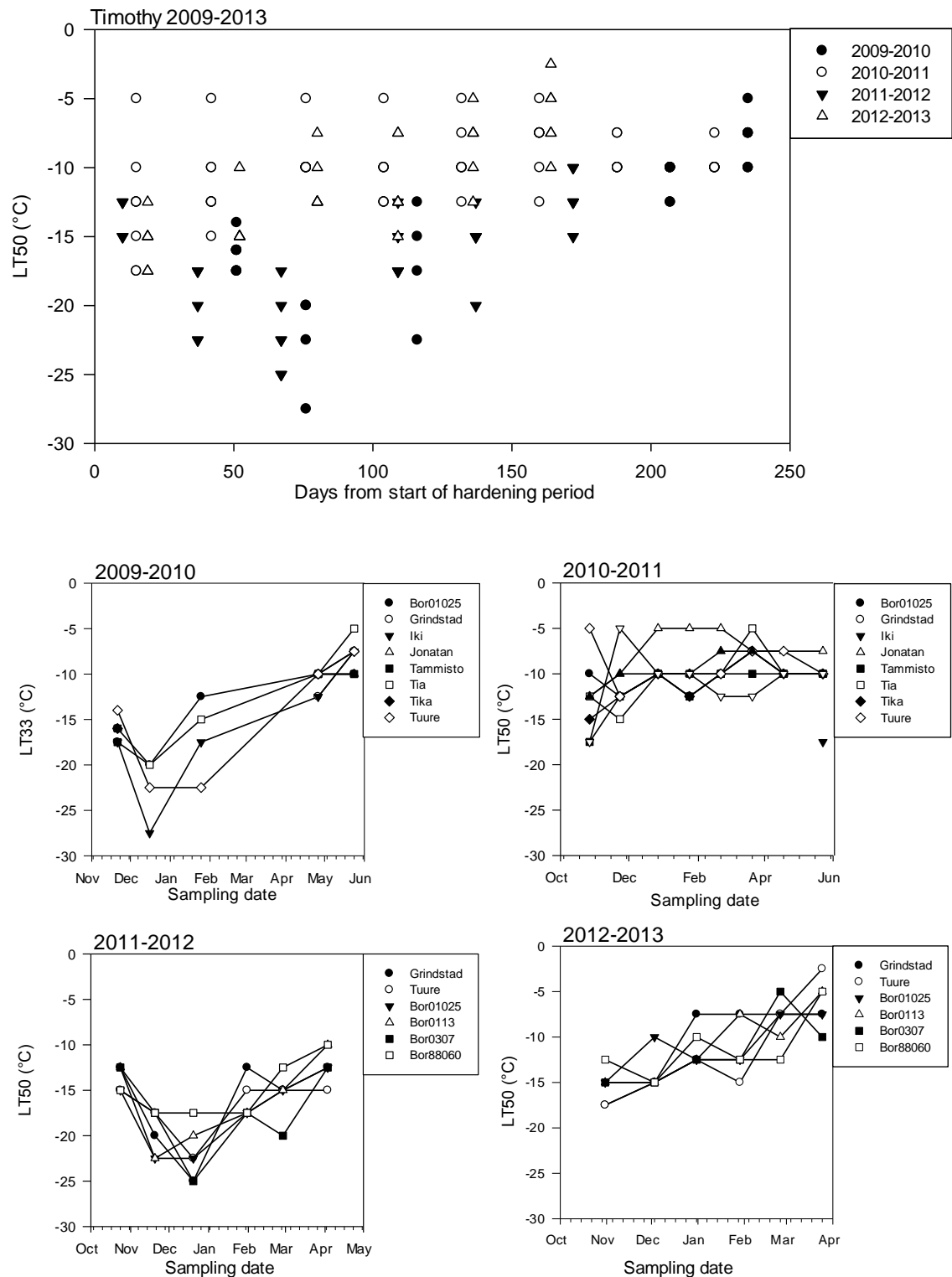
Virkajärvi, P., Pakarinen, K., Hyrkäs, M., Seppänen, M. and Bélanger, G., 2012. Tiller characteristics of timothy and tall fescue in relation to herbage mass accumulation. *Crop Science*, 52(2), pp. 970-980.

Zhang, C., Fei, S., Arora, R. and Hannapel, D.J., 2010. Ice recrystallization inhibition proteins of perennial ryegrass enhance freezing tolerance. *Planta*, 232(1), pp. 155-164.

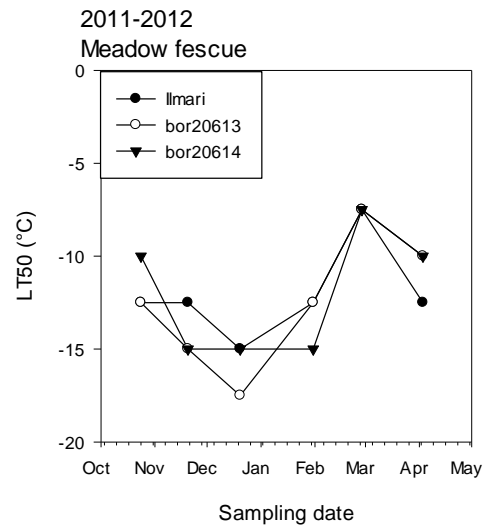
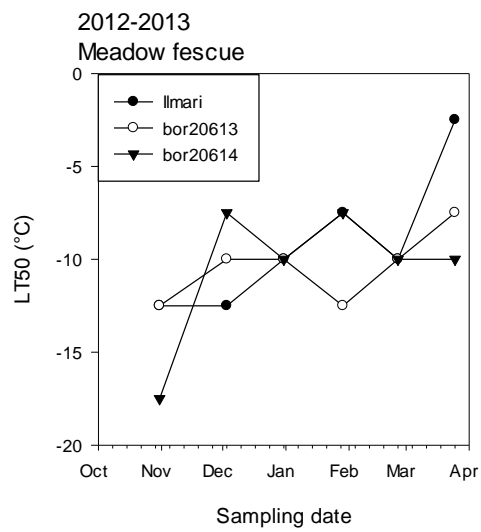
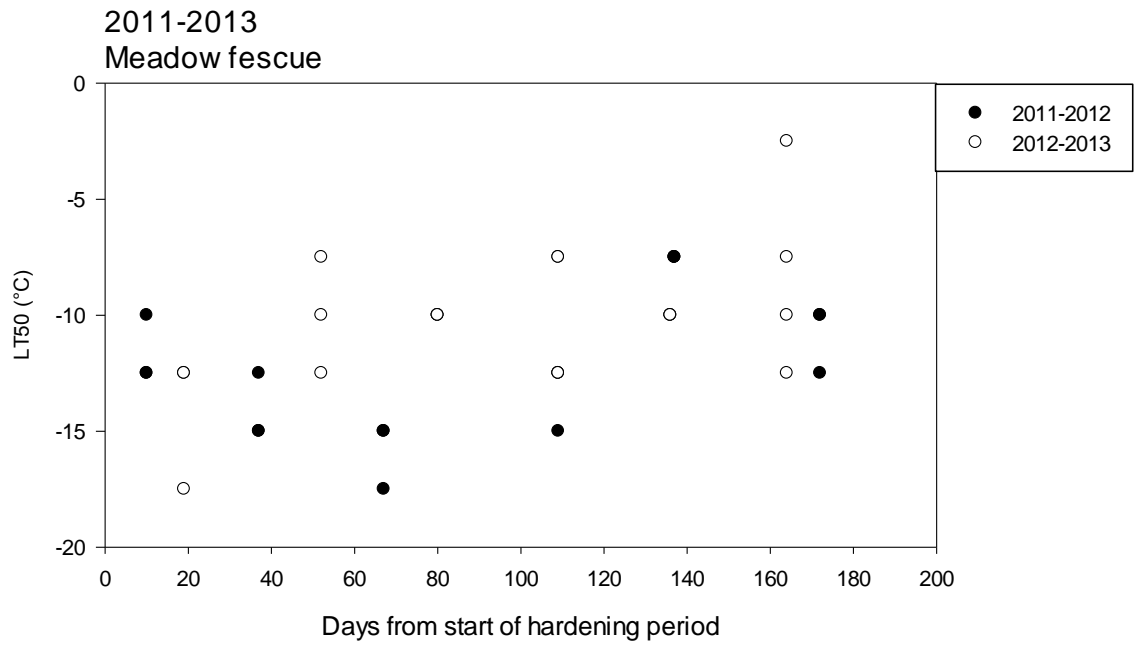
Østrem, L., Rapacz, M., Jørgensen, M. and Höglind, M., 2010. Impact of frost and plant age on compensatory growth in timothy and perennial ryegrass during winter. *Grass and Forage Science*, 65(1), pp. 15-22.

Østrem, L., Rapacz, M., Jørgensen, M. and Höglind, M., 2011. Effect of developmental stage on carbohydrate accumulation patterns during winter of timothy and perennial ryegrass. *Acta Agriculturae Scandinavica Section B–Soil and Plant Science*, 61(2), pp. 153-163.

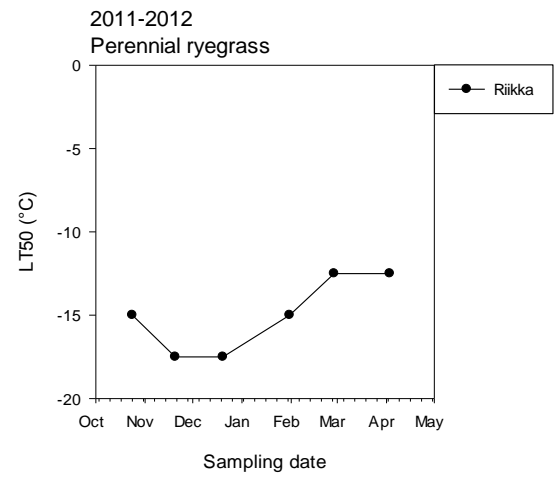
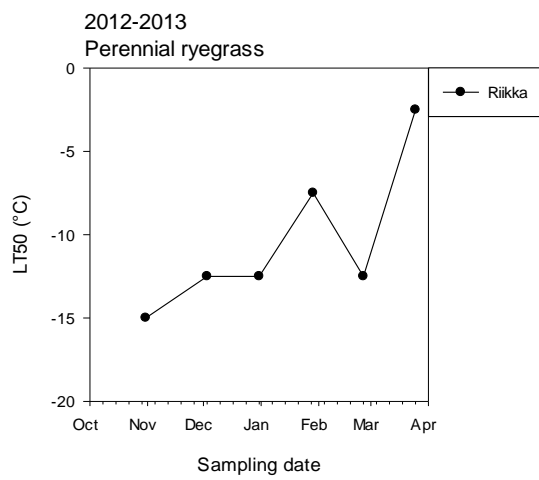
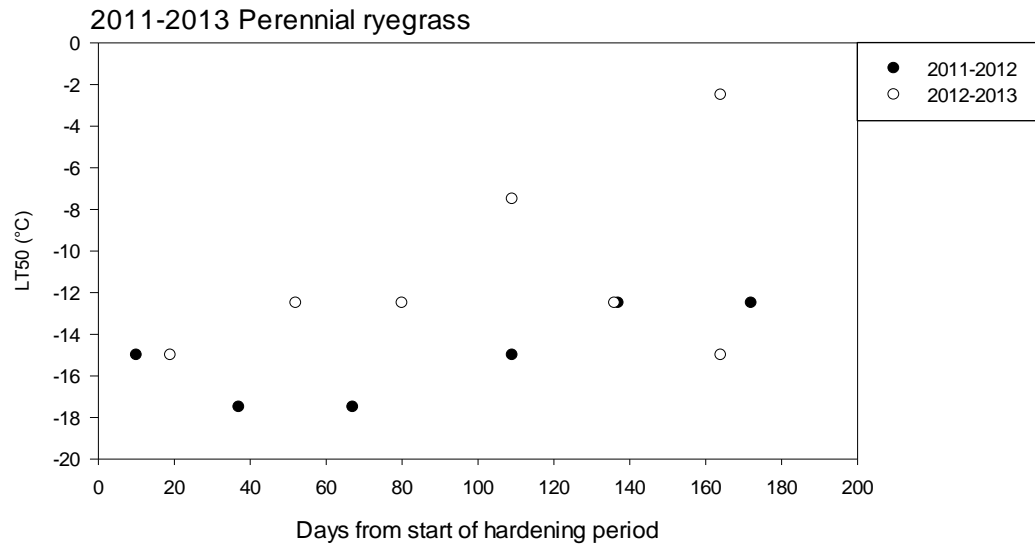
Appendices



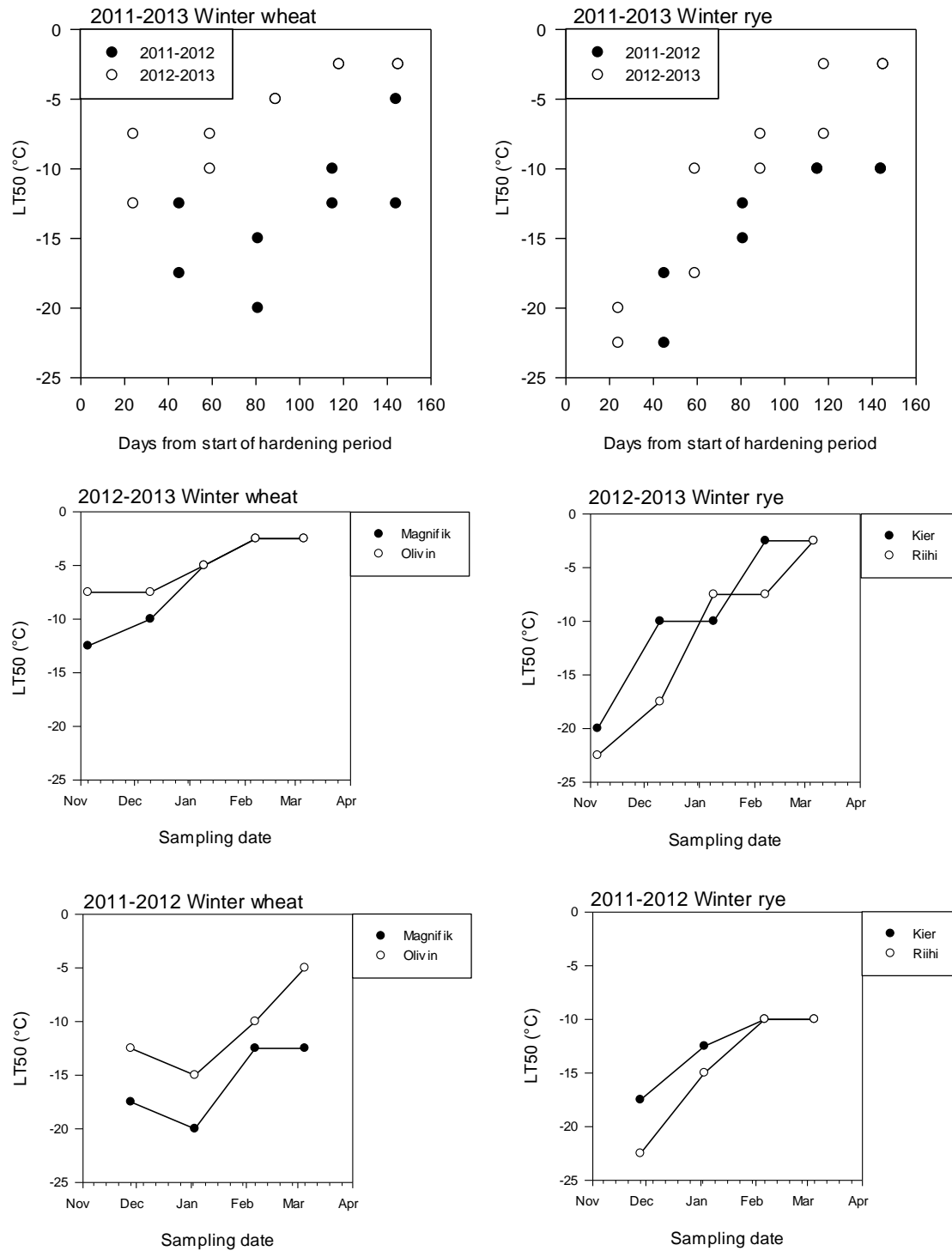
Appendix 1. LT₅₀ values of timothy cultivars. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=4. In 2009–2010 number of plants used for calculation of freezing tolerance for each temperature was three and therefore LT₃₃-value (lethal temperature for 33 % of plants) is presented.



Appendix 2. LT₅₀ values of cultivars for meadow fescue. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=4.



Appendix 3. LT₅₀ values of cultivars for perennial ryegrass. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=4.



Appendix 4. LT₅₀ values of cultivars for winter cereals. Number of plants used for calculation of LT₅₀ for each temperature: timothy n=4.